

Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

a SB298

A105

no. 47

PROCEEDINGS



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

IMPORTANT NOTICE

Preprints of papers are distributed at this meeting for the personal use of registrants only. Persons who wish to reproduce or to publish a paper must contact the author(s) for permission. U.S. copyright law specifies that copyright is vested in the individual who writes the paper or who paid to have the paper written. Papers prepared by federal employees as part of their jobs may not be copyrighted.

The papers in these proceedings have been reproduced exactly as submitted by the authors.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

PROGRAM SCHEDULE

Sunday, March 8, 1998

2:00 p.m.–7:00 p.m.	Registration	Foyer: International Ballroom
1:00 p.m.–5:00 p.m.	Table Top Exhibit Set Up	International Ballroom
6:00 p.m.–7:00 p.m.	Opening Reception	International Ballroom

Monday, March 9, 1998

7:30 a.m.–5:00 p.m.	Registration	Foyer: Madewood A&B
7:30 a.m.–8:30 a.m.	Continental Breakfast	International Ballroom
8:30 a.m.–9:30 a.m.	Opening Remarks	Madewood A&B

INVOCATION

David H. Kinard

National Cottonseed Products Association
Memphis, TN

CALL TO ORDER BY GENERAL CHAIRPERSON

Robert C. Edmonson, Senior Vice President

Applied Engineering & Science
Atlanta, GA

KEYNOTE PRESENTATION:

The Outlook for Oilseeds and Products for 1998 and Beyond

Dale F. Gustafson

Smith-Barney
Chicago, IL

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

SESSION I

Benchmarking in the Oilseed Industry

Monday, March 9, 1998

9:30 a.m. – 12:00 Noon

Madewood A & B Rooms

Session Chairperson: **Tristan Merediz**

PSI Group of Companies

Memphis, TN

- A. *Benchmarking Issues*
Mark Czarnecki, The Benchmarking Network, Houston, TX
- B. *Cooperative Oil Mill Survey*
Billy Clark, Yazoo Valley Oil Mill, Inc., Greenwood, MS
- C. *Key Components to Maximizing Yields in the Crushing Industry*
John Wright, Owensboro Grain Company, Owensboro, KY
- D. *Oil Refinery Yield Measurements*
Giles Farmer, Applied Engineering and Science, Mabank, TX



LUNCHEON

Monday, March 9, 1998

12:00 p.m.–1:45 p.m.

International Ballroom

dedicated time to view poster presentations and visit with table top exhibitors

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

SESSION II

USDA/ARS Research Emphasis on Vegetable Oils

Monday, March 9, 1998

2:00 p.m.–5:00 p.m.

Madewood A & B Rooms

Session Chairperson: **John Cherry**

USDA/ARS, Eastern Regional Research Center

Wyndmoor, PA

- E. *New Commodity Products from Soybean Through Biotechnology*
Richard F. Wilson, Soybean & Nitrogen Fixation Research Unit, ARS, USDA
Raleigh, NC
- F. *New Processes for the Conversion of Fats and Oils to Higher Value-Added Products*
Thomas A. Foglia, ERRC/ARS/USDA, Wyndmoor, PA
- G. *Oilseed and Oil Processing Research at ARS*
Peter J. Wan, SRRC/ARS/USDA, New Orleans, LA
- H. *Supercritical Fluid Technology in the Oil Processing and Conversion Industry*
Jerry W. King, NCAUR/ARS/USDA, Peoria, IL
- I. *Alternative Methods for Formulation of Food Oil Products*
Gary R. List, NCAUR/ARS/USDA, Peoria, IL

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Tuesday, March 10, 1998

7:30 a.m.–2:00 p.m. Registration

Foyer: Madewood A&B

8:00 a.m.–9:00 a.m. Continental Breakfast

International Ballroom

SESSION III

Maximizing Profit in Oilseed Crushing and Oil Refining

Tuesday, March 10, 1998

9:00 a.m.–12:00 noon

Madewood A&B Rooms

Session Chairperson: **David H. Kinard**
National Cottonseed Products Association
Memphis, TN

- J. *Minimizing Environmental Costs and Maximizing Compliance*
Michael J. Boyer, Applied Engineering & Science, Atlanta, GA
- K. *Impact of OSHA 1910.119 on Automation in Solvent Extraction Plants*
Mark B. Strube, Process Systems, Inc., Memphis, TN
- L. *Bioengineering of Oilseeds*
Frank Orthoefer, Monsanto Co., St. Louis, MO
- M. *Optimizing Tocopherol Value in Deodorizer Distillate*
Leo Walsh, Henkel Corporation, LaGrange, IL
- N. *Maximizing Profits by Minimizing Energy*
Robert Stroup, French Oil Mill Machinery Co., Piqua, OH



LUNCHEON

Tuesday, March 10, 1998

12:00 p.m.–1:45 p.m.

International Ballroom

Featured Speaker: **Ron Christiansen**,
Corporate Vice President, Chief Technology Officer, Cargill, Incorporated, Minneapolis, MN

GRAND PRIZE DRAWING AT LUNCHEON - Browning BPS Shotgun w/case

POSTER SESSION



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

POSTER SESSION

Sunday, March 8, 1998 - 6:00 p.m.–7:00 p.m.

Monday, March 9, 1998 - 12:30 p.m.–1:30 p.m.

Session Chairperson: **Armand Pepperman**

USDA, ARS, Southern Regional Research Center

New Orleans, LA

Posters will be on display throughout the conference.
Be sure to visit with the poster presenters at the times noted above.

Effect of Dry and Moist Heating on the Formation of Lipid-Protein Complexes in Soybean Products

E.A. Abd El-Motaal, H.E. Helmy, F.S. Taha, and Z.E. Shoeb

Fats and Oils Department, National Research Centre

Cairo, Egypt

Effect of Autoclaving and Storage on the Formation of Lipid-Protein Complexes in Soybean Products

H.E. Helmy, E.A. Abd El-Motaal, Z.E. Shoeb, and F.S. Taha

Fats and Oils Department, National Research Centre

Cairo, Egypt

Effect of Lipid-Protein Complexes on the Protein Fraction of Soybean

F.S. Taha, E.A. Abd El-Motaal, H.E. Helmy, and Z.E. Shoeb

Fats and Oils Department, National Research Centre

Cairo, Egypt

The Crystal and Molecular Structure of the Gossypol Triacetic Acid Clathrate

Michael K. Dowd^a and Leonard M. Thomas^b

^aSouthern Regional Research Centre, ARS, USDA, New Orleans, LA and

^bDivision of Science and Mathematics, Philipps University, Enid, OK

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Free Fatty Acids in Cottonseed and Cottonseed Products: A Literature Survey

E.J. Conkerton, A.W. Frank, and P.J. Wan

Southern Regional Research Centre, ARS, USDA

New Orleans, LA

Characterization of Lipid-Protein Complexes from Developing Tung Seeds:

Identification of Potential Intermediates in Storage Oil Biogenesis

John M. Dyer¹, Alan R. Lax¹, Dorselyn C. Chapital¹, Fuqiang Tang^{1,2}, Ding S. Shih²,
and Armand B. Pepperman¹

¹USDA-ARS-SRRC, New Orleans, LA

²Department of Biochemistry, Louisiana State University, Baton Rouge, LA

Cloning of a cDNA Homologous to Fatty Acid Ω -3 Desaturase from Developing Tung Seed

Fuqiang Tang^{1,2}, John M. Dyer¹, Alan R. Lax¹, Ding S. Shih², Dorselyn C. Chapital¹,
and Armand B. Pepperman¹

¹USDA-ARS-SRRC, New Orleans, LA

²Department of Biochemistry, Louisiana State University, Baton Rouge, LA

Fatty Acid Composition of Peanut Germplasm Collection and Structural Characterization of their Epoxy and Eicosenoic Acids

Earl G. Hammond^a, Daniel Duvick^a, Tong Wang^a, Hortense Dodo^b, and R.N. Pittman^c

^a Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, IA

^b Department of Food Science and Animal Industries, Alabama A&M University, Normal, AL

^c United States Department of Agriculture-Agricultural Research Service, Griffin, CA

Phospholipid Fatty Acid Composition and Stereospecific Distribution of Modified Soybeans

Tong Wang^a, Earl Hammond^a, and Walter R. Fehr^b

^a Department of Food Science and Human Nutrition and the Center for Crops Utilization Research, Iowa State University, Ames, IA

^b Department of Agronomy, Iowa State University, Ames, IA

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Research, Development and Technology Transfer Activities of the Center for Crops Utilization Research

L.A. Johnson and D.J. Burden

Center for Crops Utilization Research, Iowa State University

Ames, IA

New Tools for Oilseed Processing Research

L.A. Johnson and M.A. Reuber

Center for Crops Utilization Research, Iowa State University, Ames, IA

Volatile Compounds Produced During Deodorization of Soybean Oil and Their Flavor Significance

J.W. Kao, E.G. Hammond, and P.J. White

Department of Food Science and Human Nutrition, Iowa State University, Ames, IA

Development of Immunochemical Method for the Assessment of Gossypol

Xi Wang and Leslie C. Plhak

Dept. of Food Science, Louisiana Agricultural Experiment Station,

Louisiana State University, Baton Rouge, LA 70803

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

TABLE TOP EXHIBITS



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Table Top Exhibits

Alfa Laval Separation Inc., 200 South Park Blvd., Greenwood, IN 46143, USA. Alfa Laval Separation is a supplier of process equipment for the edible oil industry. Products include high speed separators, decanters, mixers and soft column deodorizers. Alfa Laval has recently introduced a new self-cleaning gas tight separator model, MiS600, designed specifically for the cottonseed oil miscella refining industry. Alfa Laval supplies complete processes, including miscella refining for cottonseed oil, degumming, neutralization, dewaxing, and deodorization.

AOCS Press, P.O. Box 3489, Champaign, IL 61826-3489, USA. AOCS Press will display books and journals related to processing interests. Among the titles on display will be the following new book releases: *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*; *Fats and Oils Handbook (Nahrungsfette und Öle)*; *World Conference on Oilseed and Edible Oils Processing: Volume I. Emerging Technologies, Current Practices, Quality Control, Technology Transfer, and Environmental Issues*, and *Volume II. Advances in Oils and Fats, Antioxidants, and Oilseed By-products*. Oilseed Conference attendees will receive discounts on these books and others.

Applied Engineering & Science, 2261 Perimeter Park Drive, Atlanta, GA 30341, USA.

Ashland Chemical/Drew Industrial Division, One Drew Plaza, Benton, NJ 07005, USA. Ashland Chemical/Drew Industrial Division is a leader in specialty chemicals and services of industrial water, fuel, and wastewater treatment. Innovative products include VALUGARD® cooling water treatment, ADVANTAGE PLUS® and Mekor® boiler water treatment. On site customized services are supplemented by computerized monitoring control and communication systems.

Bliss Industries, Inc., P.O. Box 910, Ponca City, OK 74602, USA. Manufactures over 200 different models of hammermills for customers individual needs. Ranging from 19"-52" diameter, utilizing motors from 5-600 HP. We also manufacture "The Circular Solution" to counter-flow cooling. The Bliss OP > < FLO Cooler and Pioneer Pellet Mill incorporate a well engineered design for a high degree of quality production, low maintenance and minimal downtime.

Borton, Inc., 200 E. 1st Street, Hutchinson, KS 67501, USA. Contracting and engineering company that offers to the industry conceptual facility planning, professional engineering design and drafting services, concrete auger cast-in-place deep foundation systems, new facility construction, renovation/addition to existing facilities for the food and chemical industry.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8-10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Buhler Inc., P.O. Box 9497, Minneapolis, MN 55440-9497, USA. Buhler Oil Mill Division's representatives, headquartered in Minneapolis, MN, will be available to meet with attendees about the company's line of oil milling equipment and to discuss Buhler's oilseed processing-turnkey-plant capabilities.

Continental Air Systems, P.O. Box 400, Winfield, AL 35594, USA. The Outr-A-Vac Drum filter offers maximum filtering efficiency at lower equipment cost, lower horsepower, less space, lower pressure drop, and less maintenance cost than other dry air filtering devices. Product laden air is cleaned as it passes through filter media on a rotating drum and clean air exists from the drum interior.

Crown Iron Works Company, P.O. Box 1364, Minneapolis, MN 55440, USA. Crown Iron Works specializes in design and engineering for oilseed and oil processing. Crown supplies traditional and specialty extraction equipment along with refining systems for edible oils and derivatives including methyl esters fatty acids and glycerine. Crown has offices located in the USA, United Kingdom, and Central America.

Design Corrugating, 5215 Northrup Ave., St. Louis, MO 63110, USA. Roll grinding and corrugating for cracking and flaker rolls, also new rolls.

De Smet Process & Technology, 450 Franklin Road, Suite 160, Maretta, GA 30067 USA. De Smet exhibit information on it's complete supply of oilseed extraction and refining equipment, including mechanical screw presses, solvent extraction plants, bleachers and deodorizers.

The Essmueller Company, P.O. Box 1966, Lalcurel, MS 39441-196, USA. The Essemueller Company manufactures three products in an extensive line of equipment which would be ideal for the oilseed industry. The "L" path conveyor, heavy duty flat bottom conveyer and bucket elevator are designed to meet your specialized needs. Discover 120 years of quality products and services for yourself.

Florida Industrial Filters, 305-B Scarlet Blvd., Palm Harbor, FL 34677, USA.

The French Oil Mill Machinery Company, P.O. Box 920, Piqua, OH 45356, USA. The French Oil Mill Machinery Company offers a complete line of modern and efficient oilseed preparation and solvent extraction equipment and process engineering services. The booth will feature the company's new Reflex extractor, countercurrent DTDC, flaking mill, cracking mill, ENHANSER press and prepresses.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/US

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

GTS Energy, Inc., 445 Windy Hill Rd., Marietta, GA 30060, USA. GTS offers a high-pressure natural circulation steam boiler for deodorizer applications. Unlike a conventional boiler, the GTS NUK circulates steam/water in a closed loop without need for any makeup system, blowdown, or pumps. GTS offer sizes from 100,000 Btu/hr. to 15 mm Btu/hr.

Hi Roller Conveyors, 5100 W. 12th St., Sioux Falls, SD 57107, USA. Enclosed belt conveyors with self-cleaning and self-reloading features. Designed for long life, low maintenance and dust-free grain handling.

Hutchison-Hayes Separators, Inc., P.O. Box 2965, Houston, TX 77252, USA. Parts and service for Hi-speed Vertical Disc Centrifuges, reconditioned centrifuges in shop and field service for centrifuges. Two locations to serve our customers—Houston, TX and Greenwood, IN.

Industrial & Mechanical Contractors of Memphis, 3047 Childress, Memphis, TN 38132, USA. I.M.C. with 17 years of client history takes pride in quality workmanship and timely completion of construction and shut-down project. Our ability to perform many types of work permits better control of schedules. Repeat business from satisfied customers has been our prime business and goal. See Scott Soldan or Ed Adair.

Industrial Filter & Pump Mfg. Company, 5900 Ogden Ave., Cicero, IL 60804, USA. Filtration Equipment for oil processing (crude & refining) featuring Type 122 Horizontal Tank, Vertical Leaf Filters from 50 to 2000 FT². R.H. Creager will show a bag filter vessel with disposable bags to conform to FDA regulations (absolute or nominally rated).

Interstates Electric & Engineering Company, Inc., 1520 N. Main, Sioux Center, IA 51250, USA. The Interstates' Companies offer all phases of electrical services: engineering, construction, and control systems. Let us be part of your team for your new or upgraded plant.

InterSystems, Inc., 13330 'I' St., Omaha, NE 68137, USA. InterSystems manufactures and distributes a complete line of material handling equipment for the feed, grain, and oilseed industry. Automatic samplers can be used for quality control or for official purposes. Automatic truck/rail probes can be used to obtain representative samples from inbound trucks or railcars. En-Masse Conveyors and the innovative Kleen-Masse Conveyors are available in a wide range of sizes and capacities. Bucket elevators can be constructed or painted stainless steel or galvanized. Gravity screeners can be used to clean inbound grain as well as finished product.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Laidig Industrial Systems (L.I.S. Corporation), 14535 Dragoon, Mishawaka, IN 46544, USA. Laidig Industrial Systems manufactures and constructs silo unloaders and vertical storage silos for handling various oil seed meals, hulls or pellets. The Laidig System creates a First In/First Out flow pattern within the vertical storage silo. What this means to the oil seed extraction operator: less infestation, rapid loadout of fresh meal, greater quality control, all while not worrying about the material bridging within the storage silo. Typical materials handled: soybean meal, pellets or hulls, canola meal or pellets, sunflower meal or hulls, cottonseed meal or seed hulls, undelinted or delinted cotton seed.

Law Marot Inc., 1150 Brovillette, St. Hyacinthe, Quebec J2T2G8, Canada. Law Marot offers its line of efficient and reliable heavy-duty North-American built equipment. Law Marot offers its innovative solutions for pre-cleaning the incoming product (capacity up to 20,000 bu./hr.), for hull removal achieved from a combination of sifting and gravity separation and for capacity meal sifting.

Myers Vacuum, Distillation Division, R.D. #2, Box 247A, Pine Furnace Rd., Kittanning, PA 16201, USA. Lab vacuum distillation system, separates heat sensitive, high molecular weight materials. Short path molecular vacuum still utilizes centrifugal force on a heated rotor. Shortest residence time in the industry. KF fittings for fast cleaning. Minimal glass. Scalable 2 to 2000 lbs/hour.

Oil-Dri Corporation of America, 410 N. Michigan Ave., Suite 400, Chicago, IL 60611-4211, USA. Oil-Dri Corporation of America provides innovative solutions for edible oil processing. A full-spectrum of products, including natural bleaching adsorbents, surface modified adsorbents and selective adsorbents allows Oil-Dri to meet the activity, filtration and economic needs of processors all over the globe.

Plant Maintenance Service Corporation, 3000 Fite Rd., P.O. Box 280883, Memphis, TN 38168, USA. Fabrication, installation and maintenance, specializing in oil seed processing equipment, pressure vessels, heat exchangers, condenser, and evaporators per ASME code. Conveyors, hoppers, and bins furnished and installed. Emergency services and plant turn-arounds also provided.

PSI Process Systems, Inc., 1790 Kirby Parkway, Suite 300, Memphis, TN 38138, USA. PSI representatives will present their experience in design, engineering and construction of state-of-the-art vegetable oil processing and refining plants. Emphasis will be on safety in design and construction, control systems, procurement, and single source responsibility for design/build or turnkey projects.

Resonance Instruments Ltd., Unit 13, Thorney Leys Business Park, Witney, Oxon, OX8 7GE, UK. Benchtop NMR analyzer for rapid determination of oil and moisture in seeds using simple calibrations.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

47th Oilseed Conference

March 8–10, 1998 ♦ The Doubletree Hotel ♦ New Orleans, Louisiana, USA

Roskamp Champion, 2975 Airline Circle, Waterloo, IA 50703., USA. Roskamp Champion manufactures a complete line of oil seed processing equipment. Equipment that is energy efficient robust and trouble free, for years of reliable service. The world leader with over 10,000 machines installed in over 54 countries.

Tramco, Inc., 1020 E. 19th St., Wichita, KS 67214, USA. Tramco will exhibit literature on the "worlds most complete line of chain conveyors" for the processing industry.

Trumbo, Inc., 1106 Kansas St., Memphis, TN 38106, USA. Heat-exchangers, evaporators, ASME pressure vessels, storage tanks (shop and field erected), process piping, miscellaneous structural steel towers, catwalks and supports, mechanical contracting, boiler repairs and installations, re-tube heat-exchangers, ASME code repairs and alterations, sterilizers with patented automatic doors, licensed to fab. Phillips Rod-Baffle Heat-Exch.

Westfalia Separator, Inc., 100 Fairway Ct., Northvale, NJ 07647, USA. Westfalia Separator, Inc. will feature its miscella refining separators. Westfalia Separator, a worldwide supplier of centrifuges and high intensity mixers for the vegetable oil industry, manufactures self cleaning and solid bowl separators for a wide range of capacities for use in degumming, neutralization and dewaxing.

Co-sponsored by:

AOCS

American Oil Chemists' Society

NCPA

National Cottonseed Products Association, Inc.

SRRC

Southern Regional Research Center/ARS/USDA

Benchmarking Issues

Mark Czarnecki

The Benchmarking Network
Houston, TX



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Cooperative Oil Mill Survey

William G. Clark
Yazoo Valley Oil Mill, Inc.
Greenwood, MS



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

COOPERATIVE OIL MILL SURVEY

William G. Clark
Yazoo Valley Oil Mill, Inc.
Greenwood, Mississippi

Benchmarking is a buzzword that I only started hearing last year as we were planning for the 1997 Oilseed Conference. As we began talking and planning this year, the subject of benchmarking came up again and after learning more about it, I realized that the Cooperative Cottonseed Oil Mills had been doing this since 1953. So even with my years of experience of seeing people speak up and getting saddled with having to give a talk on whatever, I still explained to the planning committee about our experience with the crusher report, so the next thing you know I'm on the program.

As I said the Cooperative Cottonseed Oil Mills have been sending information into the USDA's Agricultural Cooperative Service since 1953. This information is compiled in a format that compares these mills to each other. We sit down together, usually at our NCPA Convention, and go over the reports and interact with each other, ask questions, etc. Bruce Reynolds of the USDA is the person responsible for getting the info and putting it all together. He also meets with us. I know we have found the information and comparisons to be tremendously useful, because we can almost instantly see areas where our mill needs to improve. Probably what has made this report so useful, also, is the willing participation of almost 100% of the Cooperative Mills. This willingness to discuss the information openly with each other really adds to these reports.

What I've done is prepared overheads showing the 1989-90 processing season. I wanted to make sure that the identities of the participants remained anonymous, so I used this older report and I did delete any information that may give clues to a particular mill's identity.

Let's look at the report.

May 1991

United States
Department of
Agriculture

Agricultural
Cooperative
Service

COOPERATIVE COTTONSEED OIL MILLS

1989-90 Processing Season

Prepared by
Bruce J. Reynolds

Table 1--Laboratory analyses and product yields, 10 mills, 1989-90.

Item	1	2	3	4	5	6	7	8	9	10
mill code number										
Cottonseed:										
Tons per sample	--	99	--	87	123	87	89	69	--	--
Foreign Matter (%)	0.91	2.54	1.20	0.93	2.77	0.85	0.40	0.60	0.77	1.05
Moisture (%)	10.93	6.88	11.38	10.65	8.04	11.12	11.14	9.60	10.26	9.08
Oil (%)	17.34	18.88	17.90	17.81	18.70	18.16	17.94	17.80	17.60	17.66
Ammonia (%)	3.87	4.02	3.91	3.89	4.01	3.84	3.88	4.02	3.92	4.08
Free Fatty Acid (%)	1.16	0.45	0.67	0.64	0.76	0.86	0.80	0.40	0.69	0.43
Linters (%)	--	10.20	--	10.70	8.89	--	--	11.50	--	--
Grade	97.10	102.98	99.46	99.12	102.00	100.38	99.68	100.20	98.67	100.10
Oil:										
Refining loss (%)	9.20	7.22	8.16	7.67	--	9.15	6.75	--	--	--
Refined color {%	1.90	1.89	6.22	5.51	--	4.33	5.67	--	--	--
Meal:										
Protein (%)	42.38	40.87	41.54	40.32	40.03	40.86	41.74	40.65	42.12	41.17
Oil (%):										
No fat added	--	--	1.49	0.69	--	0.92	1.15	--	0.86	--
Fat added	3.11	2.76	--	1.99	4.18	--	--	2.67	2.84	3.25
Moisture (%)	10.20	10.82	11.47	12.10	10.32	11.37	11.36	--	10.93	--
Linters:										
Cellulose (%)	76.60	74.08	75.00	74.54	71.97	--	73.70	76.60	73.12	67.50
Moisture (%)	6.55	5.15	6.70	7.20	6.50	--	6.70	6.20	6.80	7.50
Delinted seed:										
Residual linters	2.90	2.58	2.33	2.17	2.45	--	1.50	3.40	3.20	--
Hulls:										
Oil (%)	0.53	1.48	0.78	0.66	0.99	--	0.71	1.17	0.46	0.42
Product yields(lbs):										
Crude Oil	322	--	349	345	--	347	335	--	--	--
PBSY Oil	--	320	--	--	322	--	--	315	305	304
Meal	877	915	940	914	966	906	911	901	891	942
Hulls	527	580	451	464	561	474	508	563	543	530
Linters:										
1st cut	15	32	88	51	35	62	36	43	55	50
2nd cut	39	127	113	130	108	120	123	124	136	155
mill run	127	--	--	--	--	--	--	--	--	--
motest	1	--	--	6	2	6	3	5	--	1
Total Linters	182	159	201	187	145	188	162	172	191	206
Processing loss	92	26	59	90	6	85	84	49	70	18
Total	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

Table 2--Average results of laboratory analyses and product yields per ton, 1985/86 - 1989/90

Item	1985/86 16 mills	1986/87 14 mills	1987/88 14 mills	1988/89 14 mills	1989/90 10 mills
Cottonseed:					
Tons per sample	115	104	106	98	92
Foreign Matter (%)	1.12	1.27	1.14	1.07	1.20
Moisture (%)	10.18	10.82	9.35	10.49	9.91
Oil (%)	17.58	17.19	17.75	17.36	17.98
Ammonia (%)	3.98	4.02	3.98	3.97	3.94
Free Fatty Acid (%)	0.99	0.82	0.52	0.94	0.69
Linters (%)	11.38	11.14	10.69	11.20	10.32
Grade	97.52	96.68	99.45	97.22	99.97
Oil:					
Refining loss (%)	7.99	7.4	6.80	9.11	8.03
Refined color (%)	5.41	5.03	5.13	5.41	4.25
Meal:					
Protein (%)	41.25	41.25	41.18	41.73	41.17
Oil (%):					
No fat added	1.75	1.08	1.30	1.35	1.02
Fat added	3.13	3.47	3.23	3.10	2.97
Moisture (%)	10.29	10.99	10.94	11.06	11.07
Linters:					
Cellulose (%)	75.12	72.32	74.13	75.19	73.68
Moisture (%)	6.91	7.62	6.12	6.56	6.59
Delinted seed:					
Residual linters	2.79	2.68	2.59	2.39	2.57
Hulls:					
Oil (%)	0.86	0.82	0.89	0.72	0.80
Product yields(lbs):					
Crude Oil	320	325	329	316	340
Refined Oil	309	308	328	322	313
Meal	928	935	923	912	916
Hulls	503	492	516	522	520
Linters	183	179	179	181	179

Table 3 --Costs per ton of cottonseed processed, 10 mills, 1989-90 season

Item	Mill code number									
	1	2	3	4	5	6	7	8	9	10
Capacity Use {1} **										
" {2}										
Manufacturing Costs:										
Wages & FICA	17.47	18.19	12.15	12.52	9.38	16.01	18.72	13.20	17.59	23.82
Power	8.34	5.39	4.00	6.04	4.58	8.79	7.66	5.26	10.94	8.32
Water	0.00	0.43	0.03	0.22	0.42	0.00	0.12	0.26	0.28	0.38
Fuel	3.61	2.80	3.56	2.11	2.11	3.55	2.79	2.56	2.71	4.54
Repairs	8.71	5.42	3.52	3.33	3.69	2.36	3.62	3.38	7.63	7.23
Depreciation	2.71	8.78	1.32	3.65	4.70	6.42	2.13	12.61	3.72	11.13
Mill supplies	6.46	0.93	0.81	2.00	1.64	2.18	2.38	1.54	2.43	4.41
Insurance	1.41	1.57	0.95	1.14	1.77	4.85	3.22	2.41	2.47	9.27
Laboratory	0.71	0.28	0.42	0.35	0.41	0.70	0.55	0.65	0.47	0.66
Linters bag & tie	0.83	0.74	0.70	1.07	0.57	1.37	0.42	1.47	0.71	1.53
Solvent	2.02	0.32	1.20	0.86	0.83	1.11	0.59	0.87	0.72	1.28
Miscellaneous	0.00	1.11	0.17	2.31	1.01	0.28	1.35	0.46	0.71	0.00
Total ***	52.26	45.95	28.84	35.61	31.11	47.61	43.56	44.67	50.38	72.58
Administrative costs:										
Salaries & FICA	3.45	2.46	3.44	3.46	3.20	4.01	3.61	3.50	4.07	2.16
Brokerage	0.45	0.87	0.48	1.03	0.41	0.00	0.93	0.42	0.33	0.15
Taxes & licenses	0.73	1.42	0.16	0.27	0.57	0.92	2.52	0.22	1.63	0.54
Auto & travel	0.22	0.26	0.11	0.28	0.20	0.36	0.22	0.29	0.30	0.31
Telecommunications	0.05	0.11	0.09	0.16	0.13	0.15	0.11	0.11	0.02	0.16
Dues & advertising	0.60	0.77	0.31	0.49	0.75	0.69	0.49	0.65	0.90	0.46
Audit & legal	0.02	0.11	0.04	0.16	0.10	0.95	0.16	0.31	0.16	0.15
Office supplies	0.47	0.24	0.13	0.21	0.44	0.62	0.15	0.38	0.38	0.47
Miscellaneous	0.11	0.22	0.39	0.60	0.09	0.10	0.18	0.39	0.00	0.16
Total ***	6.11	6.45	5.15	6.68	5.88	7.79	8.36	6.27	7.79	4.55
Total operating costs	58.36	52.40	33.99	42.29	36.99	55.40	51.92	50.95	58.18	77.13
Financial costs:										
Interest & exchange	4.81	8.51	1.92	4.06	0.00	6.08	5.24	3.65	3.69	11.46
Income taxes	0.96	0.00	0.00	0.13	0.83	0.19	0.06	-0.42	0.00	0.13
Total ***	5.77	9.27	1.92	4.19	0.83	6.27	5.30	3.23	3.69	11.59

* Average of multiple plants.

** Capacity use: (1) processing days / 330 days, (2) tons processed / (daily capacity X 330 days).

*** Rounding differences make some totals differ from the sum of their per ton columns by .01.

Fig. 1- Manufacturing Costs per Ton
10 mills, 1989-90

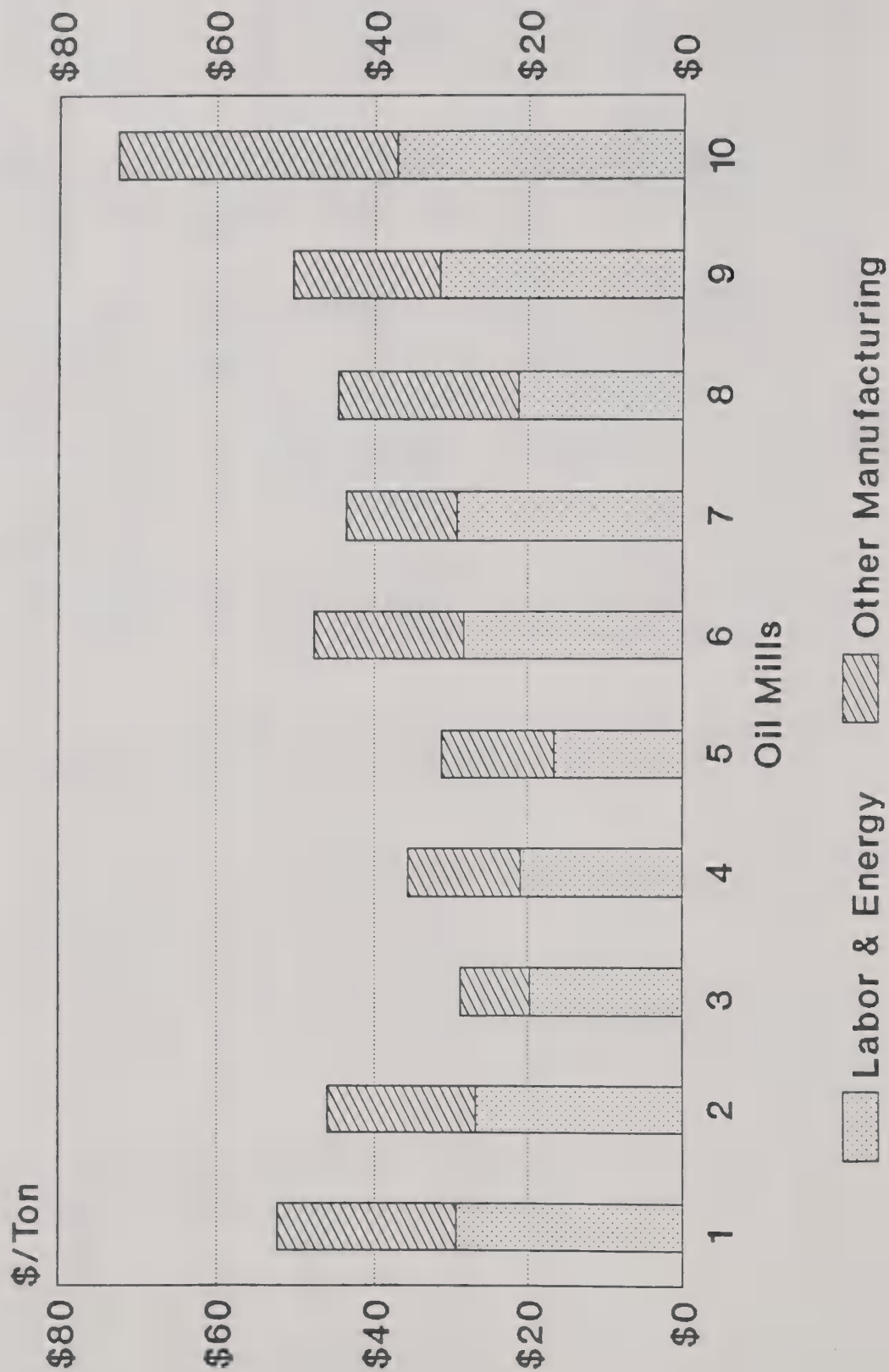


Fig. 2 - Costs per Ton
10 mills, 1989-90

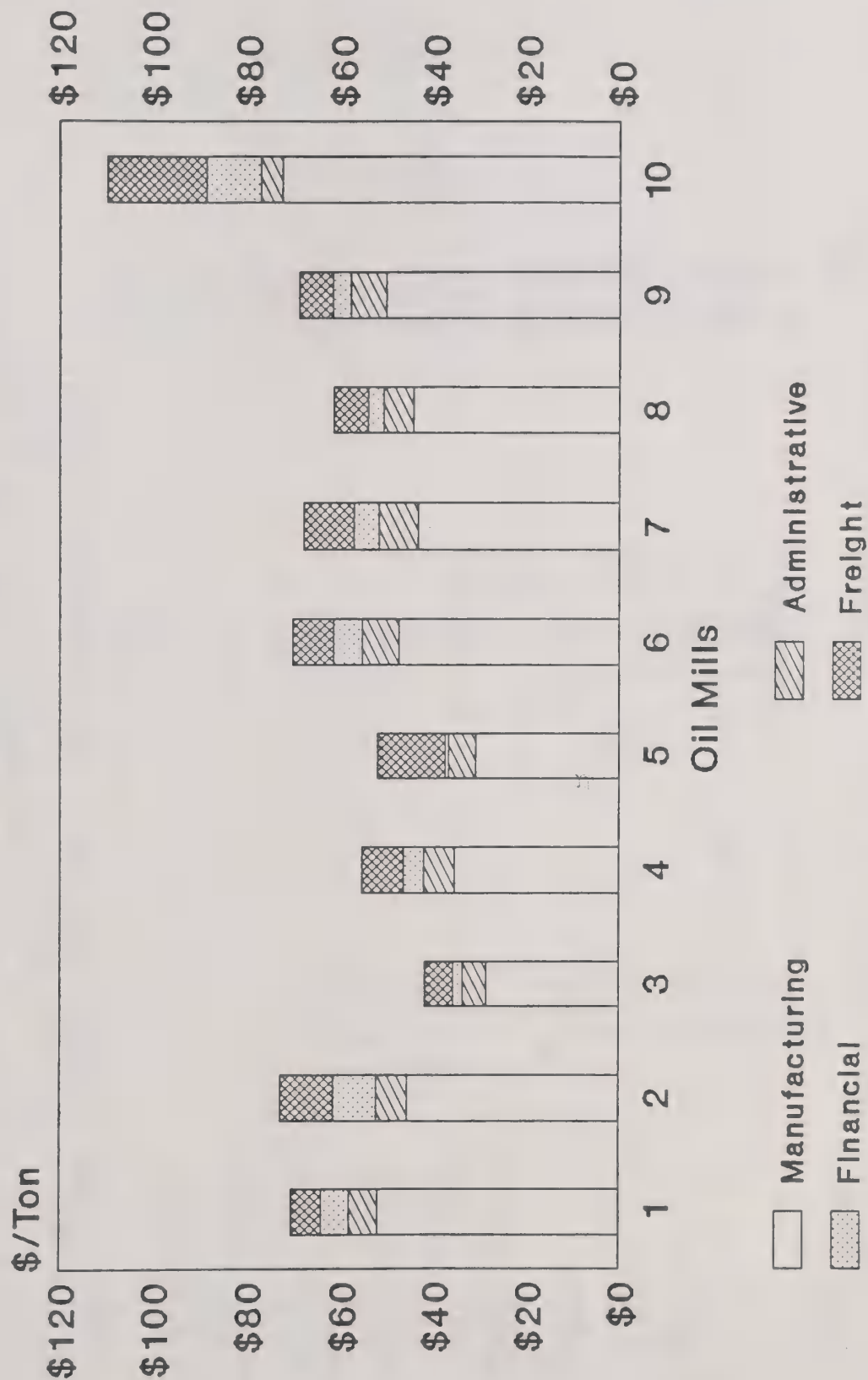


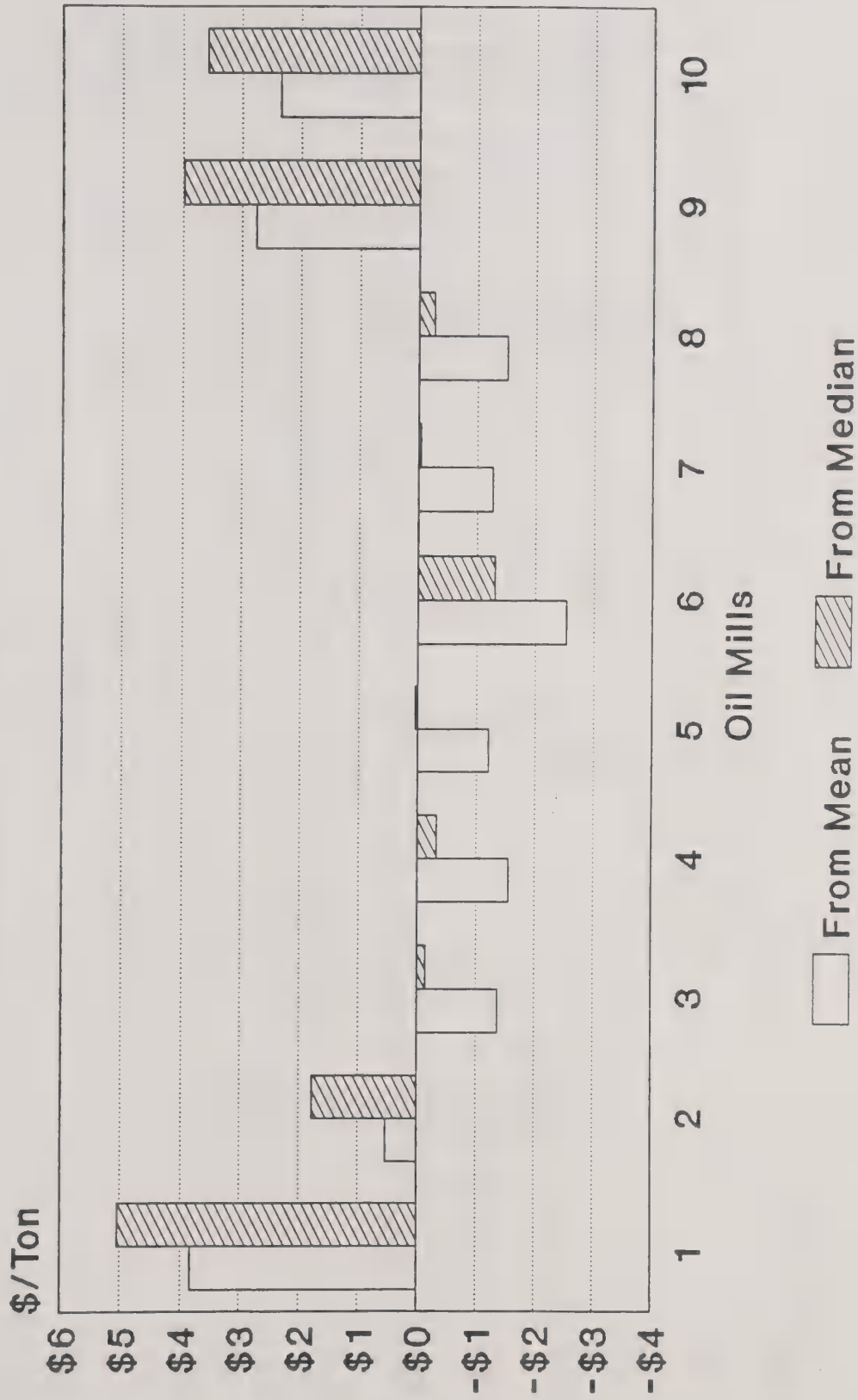
Table 4 --Average costs per ton, 10 mills, 1989-90

Item	Weighted Average	10 mills Unweighted Average	Median
Tons Processed *	1,198,689	119,869	85,717
Capacity " Use {1} **	83%	71%	78%
{2}	71%	66%	70%
Manufacturing Costs:			
Wages & FICA	15.82	15.91	16.74
Power	6.67	6.93	7.01
Water	0.19	0.21	0.25
Fuel	3.05	3.03	2.79
Repairs	5.45	4.89	3.66
Depreciation	5.03	5.72	4.21
Mill supplies	2.84	2.48	2.15
Insurance	1.99	2.91	2.09
Laboratory	0.51	0.52	0.51
Linters bag & tie	0.85	0.94	0.78
Solvent	1.09	0.98	0.89
Miscellaneous	0.69	0.74	0.65
Total	44.18	45.26	45.31
Administrative costs:			
Salaries & FICA	3.34	3.34	3.48
Brokerage	0.58	0.51	0.44
Taxes & licenses	0.91	0.90	0.65
Auto & travel	0.25	0.25	0.27
Telecommunications	0.10	0.11	0.11
Dues & advertising	0.63	0.61	0.62
Audit & legal	0.16	0.22	0.16
Office supplies	0.35	0.35	0.38
Miscellaneous	0.23	0.22	0.17
Total	6.55	6.50	6.36
Total operating costs	50.73	51.76	52.16
Financial costs:			
Interest & exchange	4.95	4.94	4.55
Income taxes	0.47	0.19	0.09
Total	5.43	5.20	4.86

* Tons processed under weighted average is the volume of all mills.
 ** Capacity use: {1} processing days / 330 days, {2} tons processed / (daily capacity X 330 days)

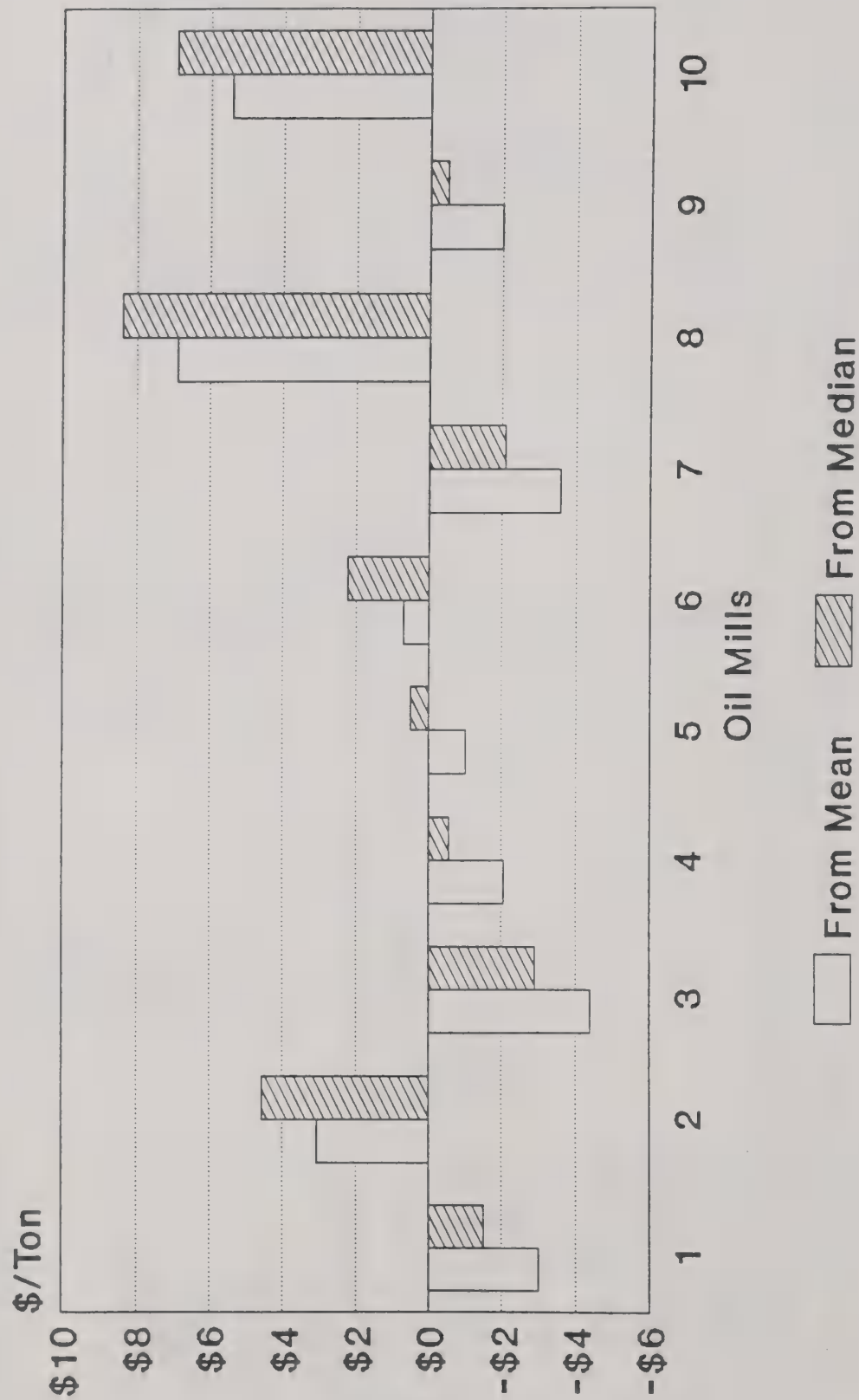
Note: Unweighted average is the simple average, the sum of 10 mills, volumes divided by 10. Weighted averages are weighted by respective volumes processed. Median values are a mid-point, for example, the sum of the 5th and 6th highest per ton cost divided by 2.

Fig. 3 - Repairs Cost Differences
Differences from Mean and Median
1989-90



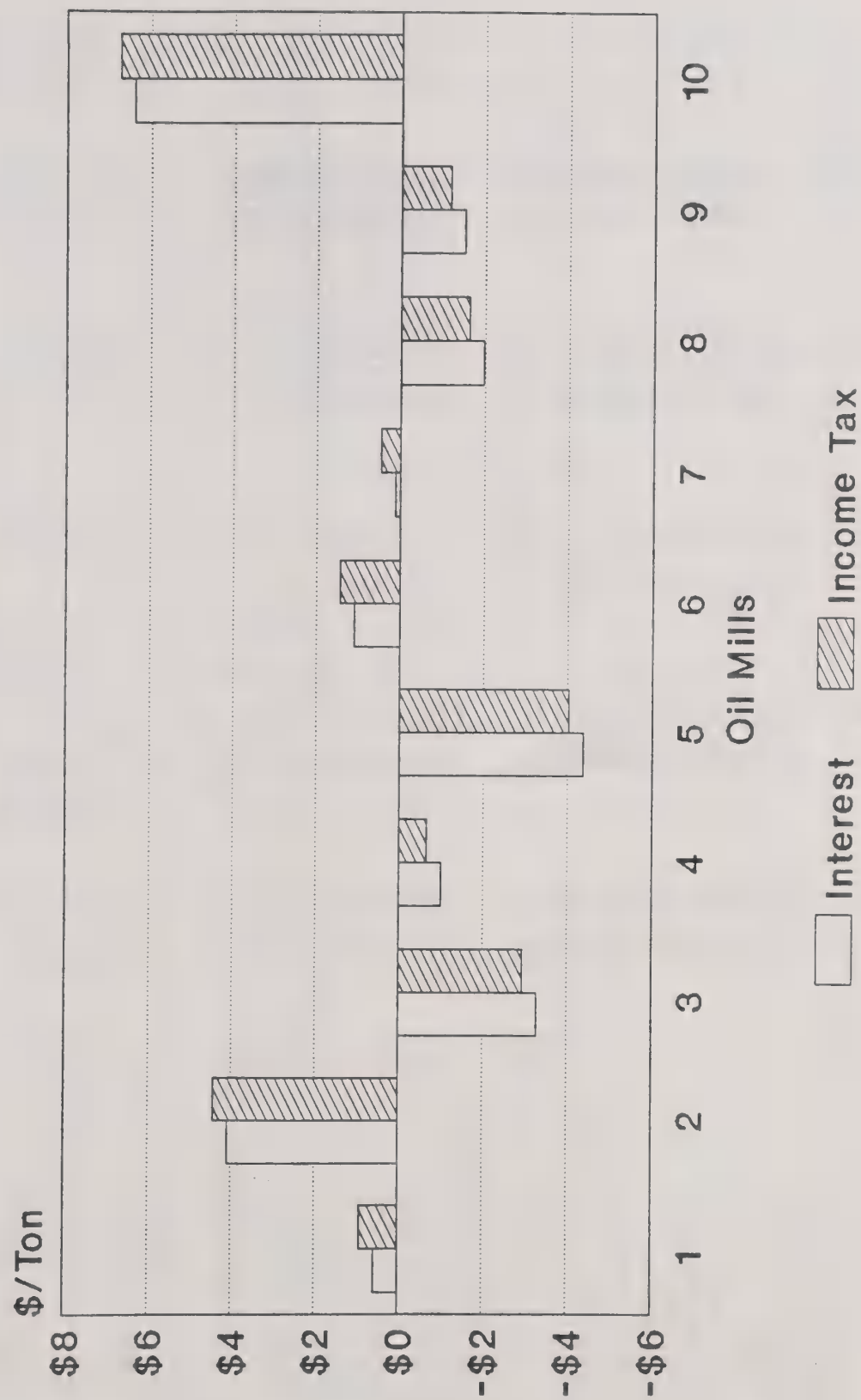
6 mills below mean

Fig. 4 - Depreciation Cost Differences
Differences from Mean and Median
1989-90



6 mills below mean

Fig. 5 - Financial Cost Differences
Differences from Mean and Median
1989-90



5 mills below mean

Table 5 --Average costs per ton, seasons 1985/86-1989/90.

Item	1985/86 16 mills	1986/87 14 mills	1987/88 14 mills	1988/89 14 mills	1989/90 10 mills
Capacity "Use {1} {2}	71% --	69% 66%	84% 79%	85% 81%	71% 66%
Manufacturing Costs:					
Wages & FICA	16.06	15.77	13.80	14.63	15.91
Power	7.45	7.47	6.83	6.98	6.93
Water	0.10	0.13	0.11	0.12	0.21
Fuel	4.10	3.56	2.80	3.11	3.03
Repairs	4.86	4.83	5.39	5.92	4.89
Depreciation	4.57	5.05	3.51	3.62	5.72
Mill supplies	2.05	2.61	1.52	2.66	2.48
Insurance	1.50	2.17	1.91	2.01	2.91
Laboratory	0.34	0.42	0.48	0.61	0.52
Linters bag & tie	0.73	0.65	0.63	0.73	0.94
Solvent	2.08	1.32	1.31	1.30	0.98
Miscellaneous	0.60	0.38	0.29	0.27	0.74
Total	44.47	44.37	38.58	41.99	45.26
Administrative costs:					
Salaries & FICA	3.11	3.28	3.28	2.87	3.34
Brokerage	0.56	0.50	0.51	0.48	0.51
Taxes & licenses	0.78	0.95	0.75	0.74	0.90
Auto & travel	0.24	0.26	0.21	0.18	0.25
Telecommunications	0.10	0.10	0.08	0.08	0.11
Dues & advertising	0.46	0.54	0.47	0.53	0.61
Audit & legal	0.17	0.18	0.17	0.15	0.22
Office supplies	0.29	0.41	0.33	0.34	0.35
Miscellaneous	0.73	0.72	0.37	0.27	0.22
Total	6.43	6.94	6.19	5.63	6.50
Total operating costs	50.90	51.31	44.77	47.62	51.76
Financial costs:					
Interest & exchange	3.25	3.75	3.46	4.72	4.94
Income taxes	0.01	0.05	0.06	0.21	0.19
Total	3.26	3.80	3.52	4.93	5.20

Table 6 --Operating results per ton processed and received, 10 mills, 1989-90

Item	1	2	3	Mill code number					8	9	10
				4	5	6	7				
Unit prices											
Crude oil (\$/lb)	20.60	--	21.03	21.49	--	21.13	20.67	--	24.00	--	--
PBSY (\$/ton)	158.05	162.10	157.39	150.62	25.41	158.60	159.50	24.00	177.68	24.18	22.76
Meals (\$/ton)	44.40	53.95	46.93	52.97	180.93	52.00	49.50	171.72	167.75	167.75	189.85
Hulls (\$/lb)	21.81	20.68	21.18	20.16	68.94	20.67	21.31	20.19	35.91	35.91	55.00
Lint (\$/lb)	22.00	21.73	21.42	22.42	21.26	21.75	21.59	21.00	18.73	18.73	20.09
1st cut	22.08	20.42	20.99	19.58	22.63	20.57	21.29	20.00	20.04	20.04	20.16
2nd cut	22.40	--	--	--	21.04	--	--	--	18.20	18.20	20.16
mill run	11.23	--	--	--	--	--	--	--	--	--	--
motes	167.51	172.29	159.13	161.01	9.07	11.41	12.75	18.00	--	--	5.70
Wholesale (\$/ton)					190.00	173.57	157.46	138.24	158.55	158.55	159.23
Gross sales receipts											
Oil	70.65	109.62	73.53	72.06	85.60	73.32	72.87	95.01	73.87	73.87	88.04
Meal	70.56	72.34	73.88	68.81	88.72	71.84	77.13	79.82	74.96	74.96	82.50
Hulls	11.77	19.31	10.84	13.89	20.79	12.32	15.84	17.34	9.74	9.74	20.55
Lint	42.91	36.53	42.59	38.83	33.46	38.52	37.38	37.78	37.65	37.65	43.30
Total	195.89	237.79	200.84	193.60	228.58	196.00	203.23	229.95	196.22	196.22	234.39
Deductions											
Freight on seed	6.45	11.24	6.14	8.95	14.35	8.58	10.78	7.40	7.05	7.05	21.17
Operating costs	58.36	52.40	33.99	42.29	36.99	55.40	51.92	50.95	58.18	58.18	77.13
Financial costs	5.77	9.27	1.92	4.19	0.83	6.27	5.30	3.23	3.69	3.69	11.59
Total	70.58	72.91	42.05	55.43	52.17	70.25	68.00	61.58	68.92	68.92	109.89
Net returns											
Net sales	125.31	164.88	158.80	138.17	176.41	125.74	135.23	168.37	127.30	127.30	124.50
Other income	0.21	0.68	1.20	0.97	1.19	2.28	1.77	0.86	0.57	0.57	12.13
Total	125.52	165.56	160.00	139.14	177.60	128.02	137.00	169.23	127.87	127.87	136.63
Adjustments ***	5.55	-1.17	-11.31	6.46	-18.27	13.11	0.30	3.56	6.89	6.89	-2.39
Total **	131.07	164.39	148.69	145.60	159.33	141.13	137.30	172.79	134.76	134.76	134.24
Available to distribute											
Initial advance	97.10	144.39	93.33	99.57	148.01	106.74	101.03	126.81	108.02	108.02	128.45
Balance	33.97	19.99	55.36	46.03	11.32	34.39	36.27	45.98	26.74	26.74	5.79
Total **	131.07	164.39	148.69	145.60	159.33	141.13	137.30	172.79	134.76	134.76	134.24

* Average of multiple plants.

** Per ton received.

*** Converting to per ton received units, with cottonseed sales revenue and inventory changes.

Table 7 -- Average operating results per ton, 10 mills, 1989-90

Item	Weighted Average	10 mills Unweighted Average	Median
Unit prices			
Crude oil (\$.01/lb)	20.87	20.98	21.03
PBSY (\$.01/lb)	25.67	24.64	24.18
Meal (\$/ton)	162.36	166.25	160.80
Hulls (\$/ton)	50.46	53.13	52.49
Lint (\$.01/lb)	20.56	20.61	20.68
1st cut	21.73	21.47	21.66
2nd cut	20.31	20.23	20.29
motes	7.47	11.67	11.41
Wholesale (\$/ton) *	164.42	163.70	160.12
Gross sales receipts			
Oil	82.99	81.46	73.70
Meal	74.45	76.06	74.42
Hulls	14.88	15.24	14.87
Lint	39.48	38.90	38.15
Total	211.80	211.65	202.03
Deductions			
Freight on seed **	9.06	10.21	8.76
Operating costs	50.73	51.76	52.16
Financial costs	5.43	5.21	4.75
Total	65.22	67.18	68.46
Net returns			
Net sales	146.58	144.47	136.70
Other income	1.09	2.19	1.08
Total	147.67	146.66	138.07
Adjustments ***	-0.14	0.27	5.30
Total **	147.53	146.93	143.37
Available to distribute			
Initial advance	114.38	115.35	107.38
Balance	33.16	31.59	34.18
Total **	147.53	146.93	143.36

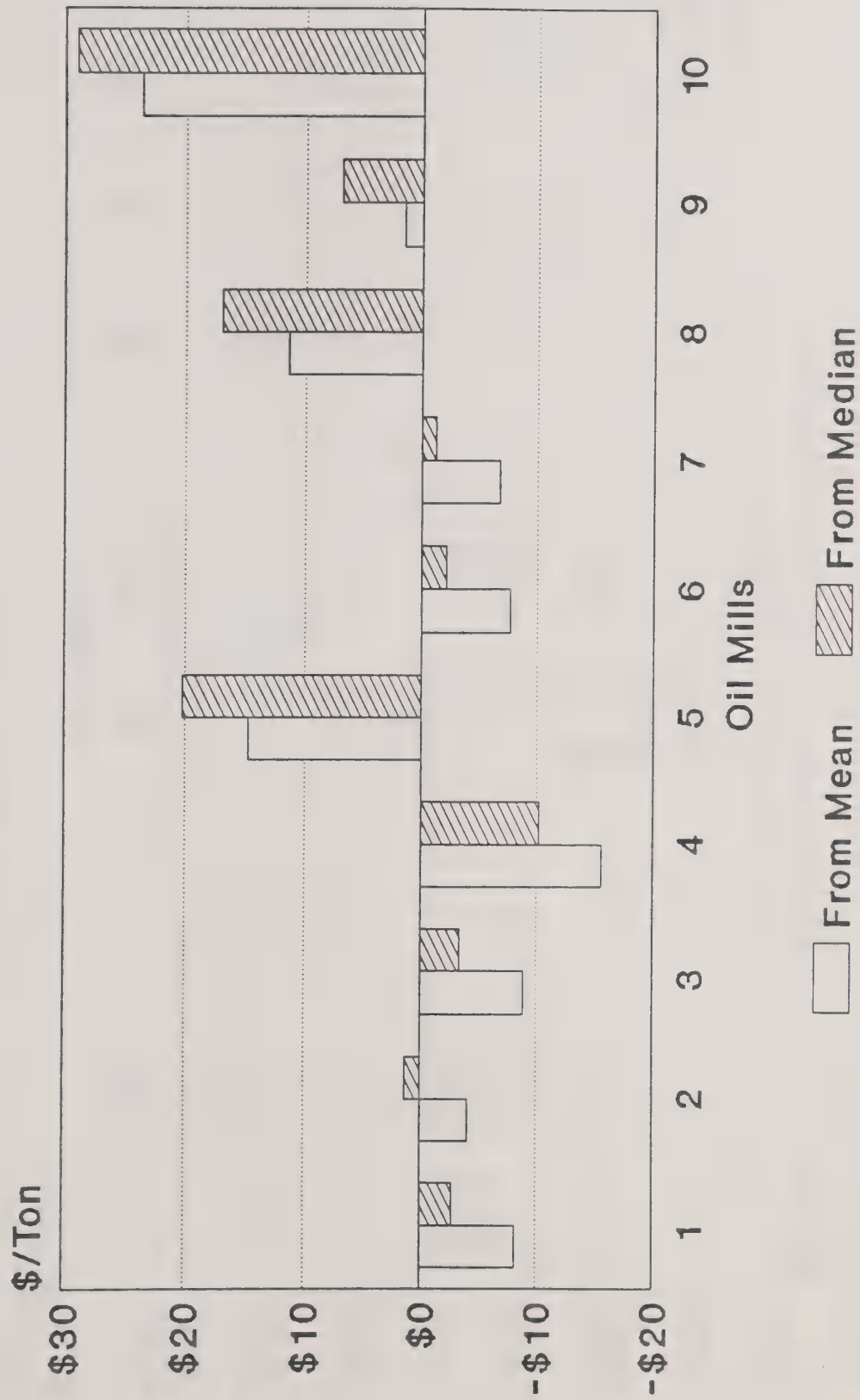
* Weighted by tons sold and products prices weighted by tons processed.

** Per ton received.

*** Inventory and freight adjustments.

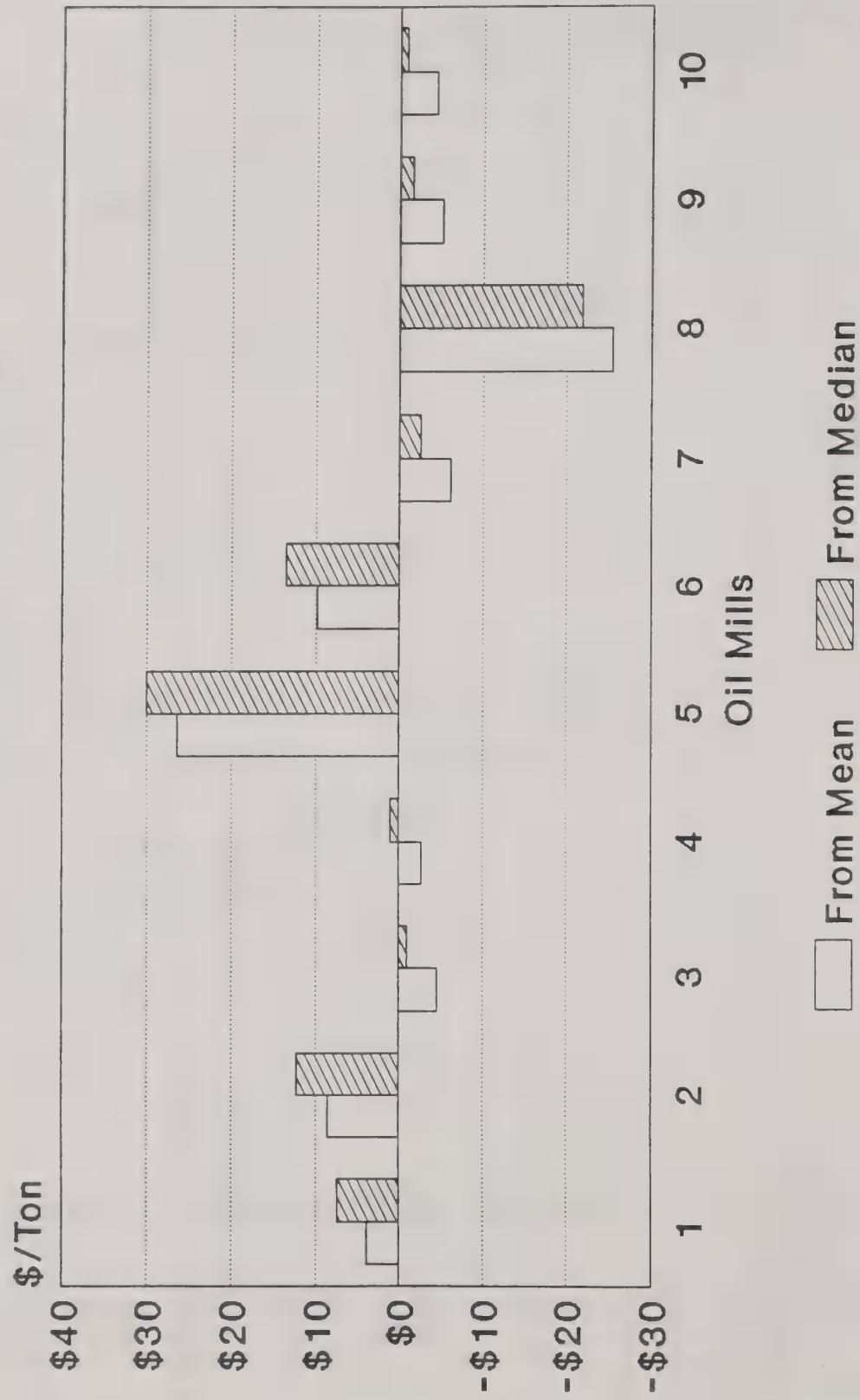
Note: See corresponding note to table 4 for explanation of weighted average and median.

Fig. 6 - Meal Price Differences
Differences from Mean and Median
1989-90



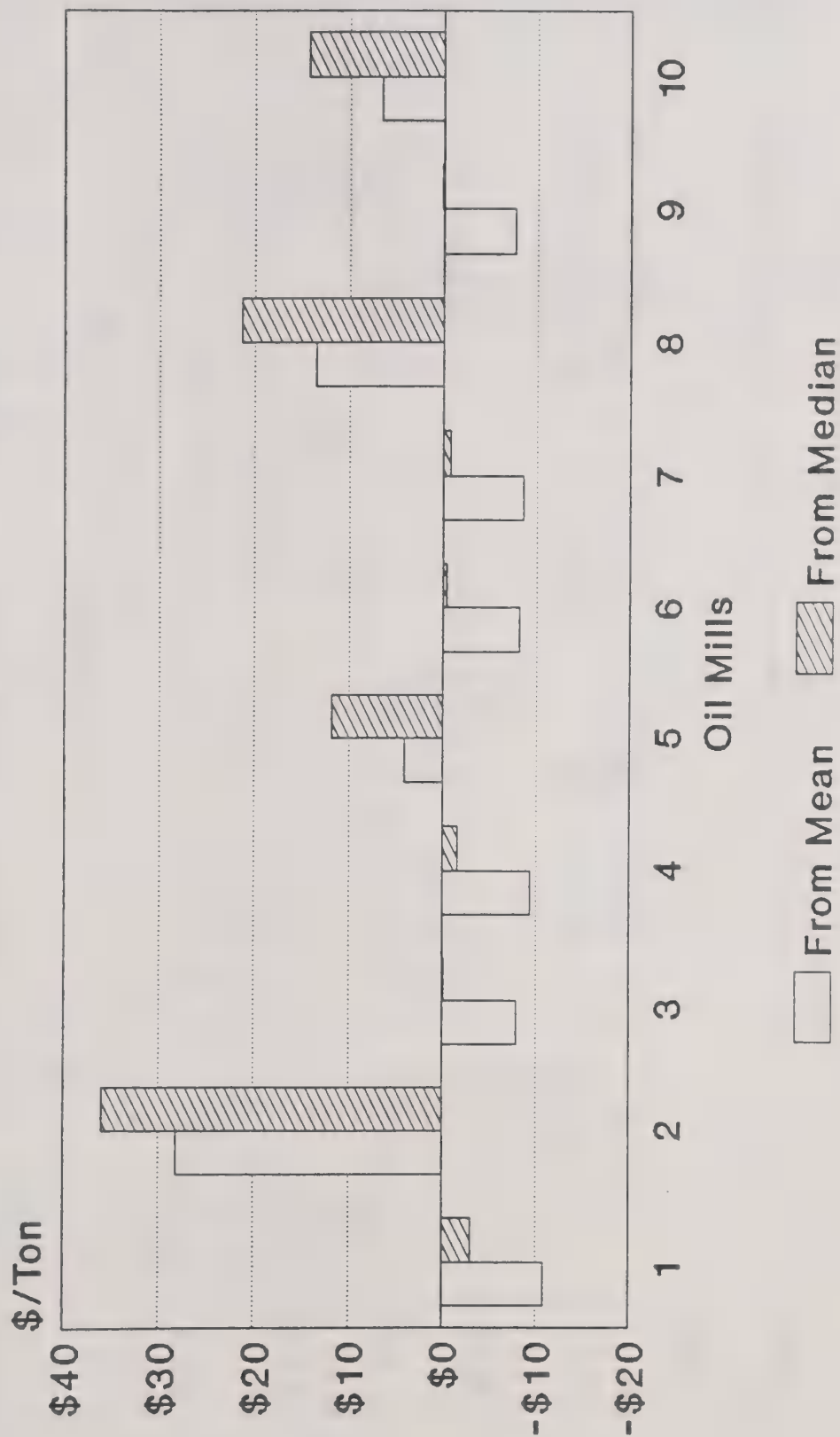
4 mills above mean

Fig. 7 - Wholeseed Price Differences
Differences from Mean and Median
1989-90



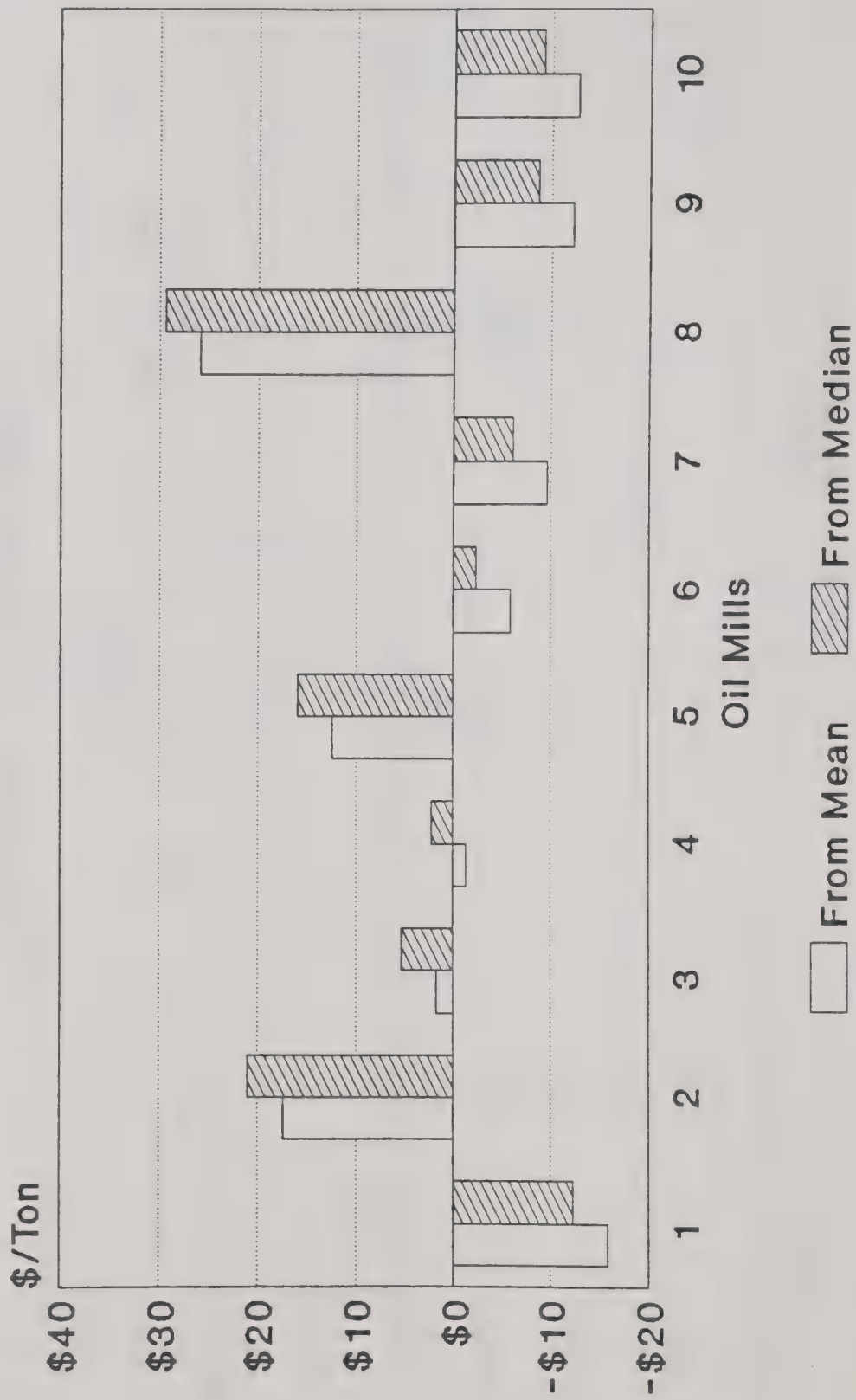
4 mills above mean

Fig. 8 - Oil Sales Differences
Differences from Mean and Median
1989-90



4 PBSY mills above mean, 5 above
or at median

Fig. 9 - Total Distribution Differences
Differences from Mean and Median
1989-90



4 mills above mean

Table 8 --Average operating results per ton, seasons 1985/86-1989/90

Item	1985/86 16 mills	1986/87 14 mills	1987/88 14 mills	1988/89 14 mills	1989/90 10 mills
Unit prices					
Crude oil (\$.01/lb)	18.90	15.24	18.83	21.40	20.98
PBSY oil (\$.01/lb)	23.59	25.23	20.05	23.52	24.64
Meal (\$/ton)	115.92	137.31	150.13	189.10	166.25
Hulls (\$/ton)	28.58	47.36	29.22	34.27	53.13
Lint (\$.01/lb)	8.91	10.41	13.79	15.35	20.61
1st cut	12.28	15.06	14.93	16.56	21.47
2nd cut	8.01	8.53	11.67	14.65	20.23
motes	4.76	6.94	10.82	8.23	11.67
Wholeseed (\$/ton)	104.88	115.52	123.97	151.18	163.70
Gross sales receipts					
Oil	64.56	58.26	64.11	70.78	81.46
Meal	53.31	62.89	69.82	85.10	76.06
Hulls	7.72	12.13	8.47	9.40	15.24
Lint	15.98	18.64	24.78	27.51	38.90
Total	141.56	151.93	167.19	192.80	211.65
Deductions					
Freight on seed *	8.34	7.94	7.62	8.80	10.21
Operating costs	49.84	51.31	44.81	47.62	51.76
Financial costs	3.65	3.80	3.39	4.93	5.21
Total	61.83	63.05	55.82	61.34	67.18
Net returns					
Net sales	79.73	88.88	111.36	131.45	144.47
Other income	6.83	2.79	1.17	0.97	2.19
Total	86.56	91.68	112.54	132.42	146.66
Adjustments **	-5.24	4.70	-0.09	1.83	0.27
Total *	81.33	96.38	112.45	134.24	146.93
Available to distribute					
Initial advance	61.00	70.12	77.88	107.54	115.35
Balance	20.32	26.26	34.57	26.70	31.59
Total *	81.33	96.38	112.45	134.24	146.93

* Per ton received.

** Inventory and freight adjustments.

Fig. 10 - Nominal Oil Prices

Average annual price
1986 to 1990

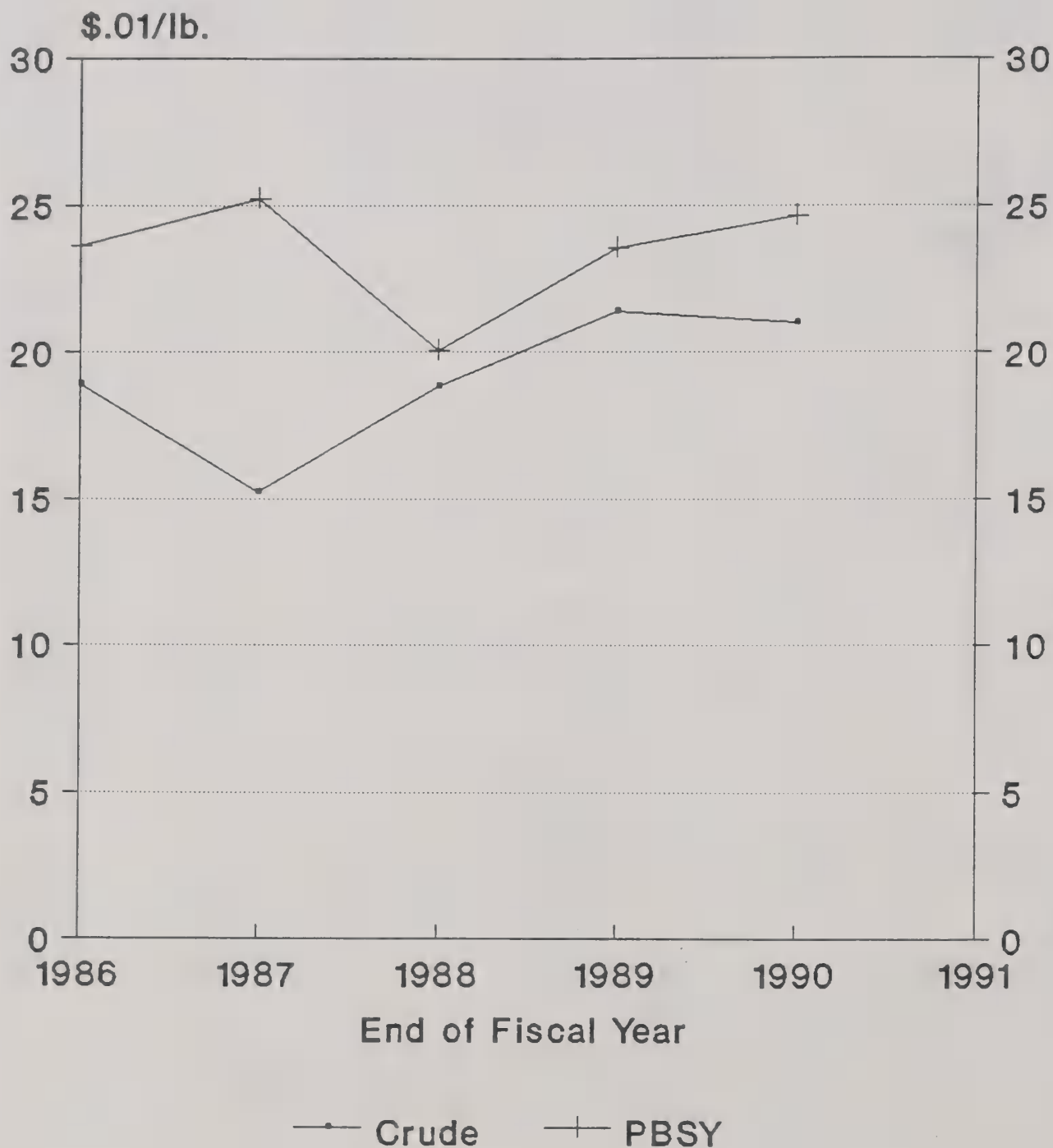


Fig. 11 - Nominal Meal Prices

Average annual price
1986 to 1990

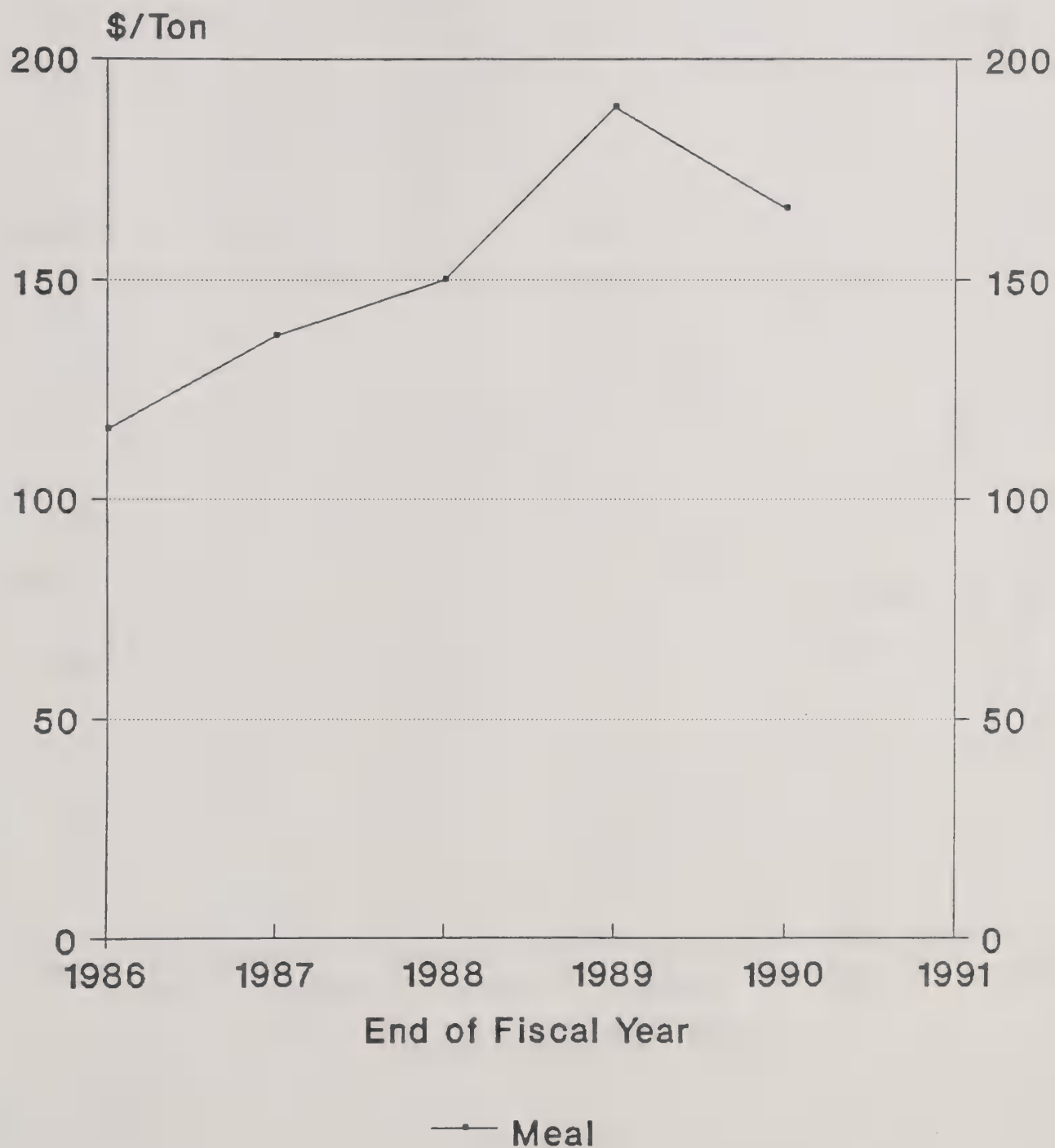


Fig. 12 - Nominal Hull Prices

Average Annual Price
1986 to 1990

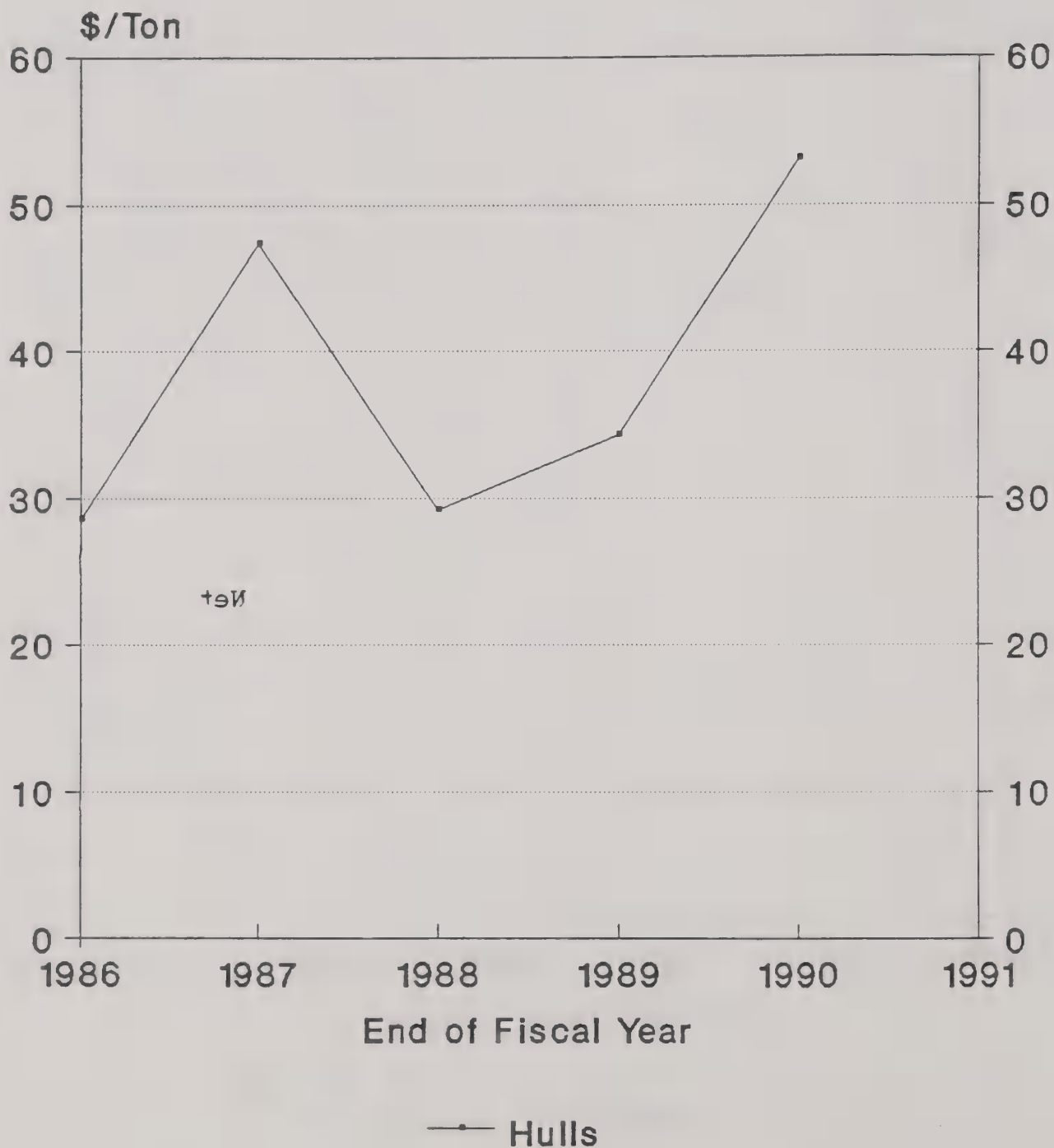
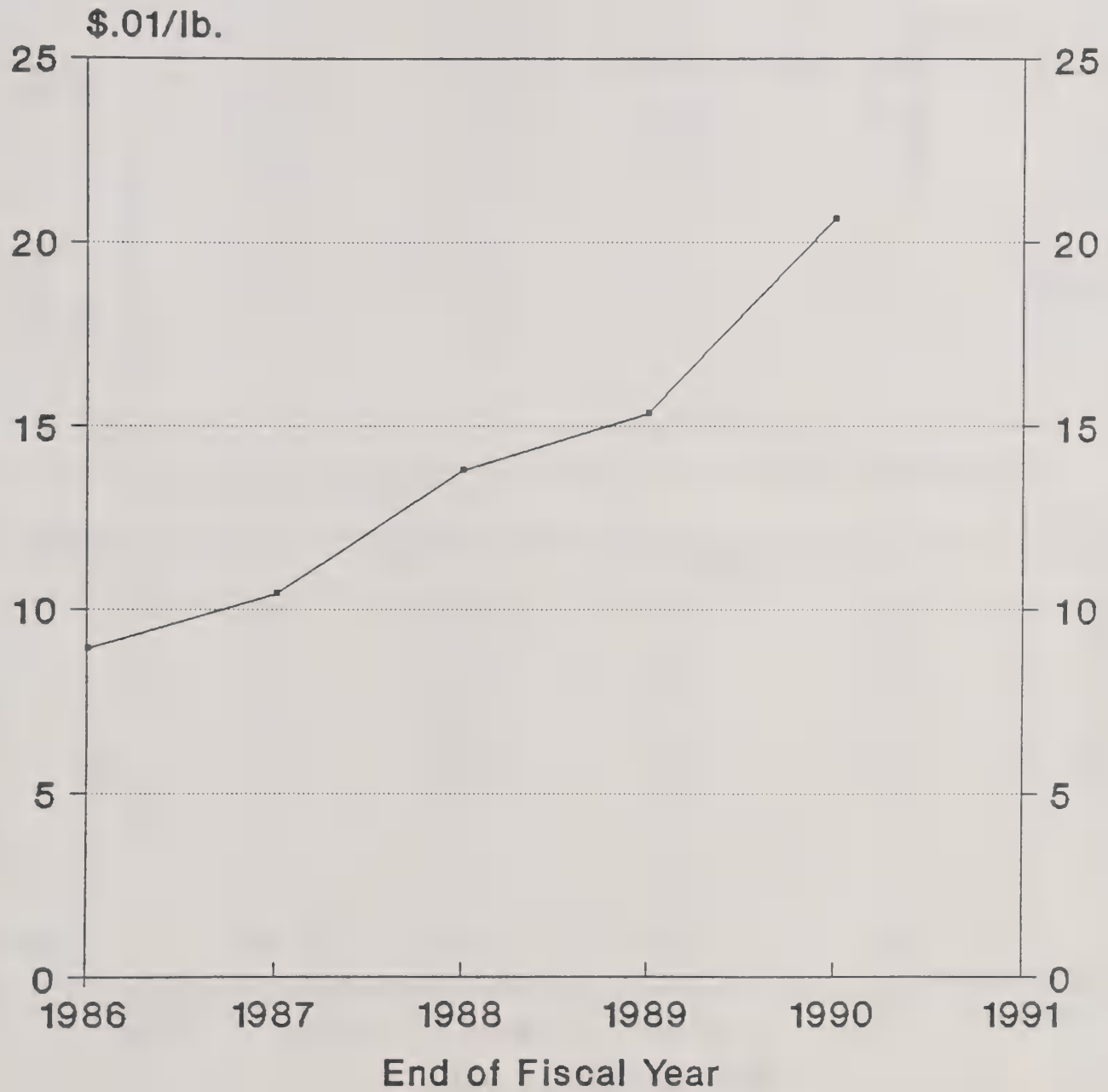


Fig. 13 - Nominal Linters Prices

Average annual price
1986 to 1990



— Linters

Fig. 14 - Nominal Wholeseed Prices

Average annual price
1986 to 1990

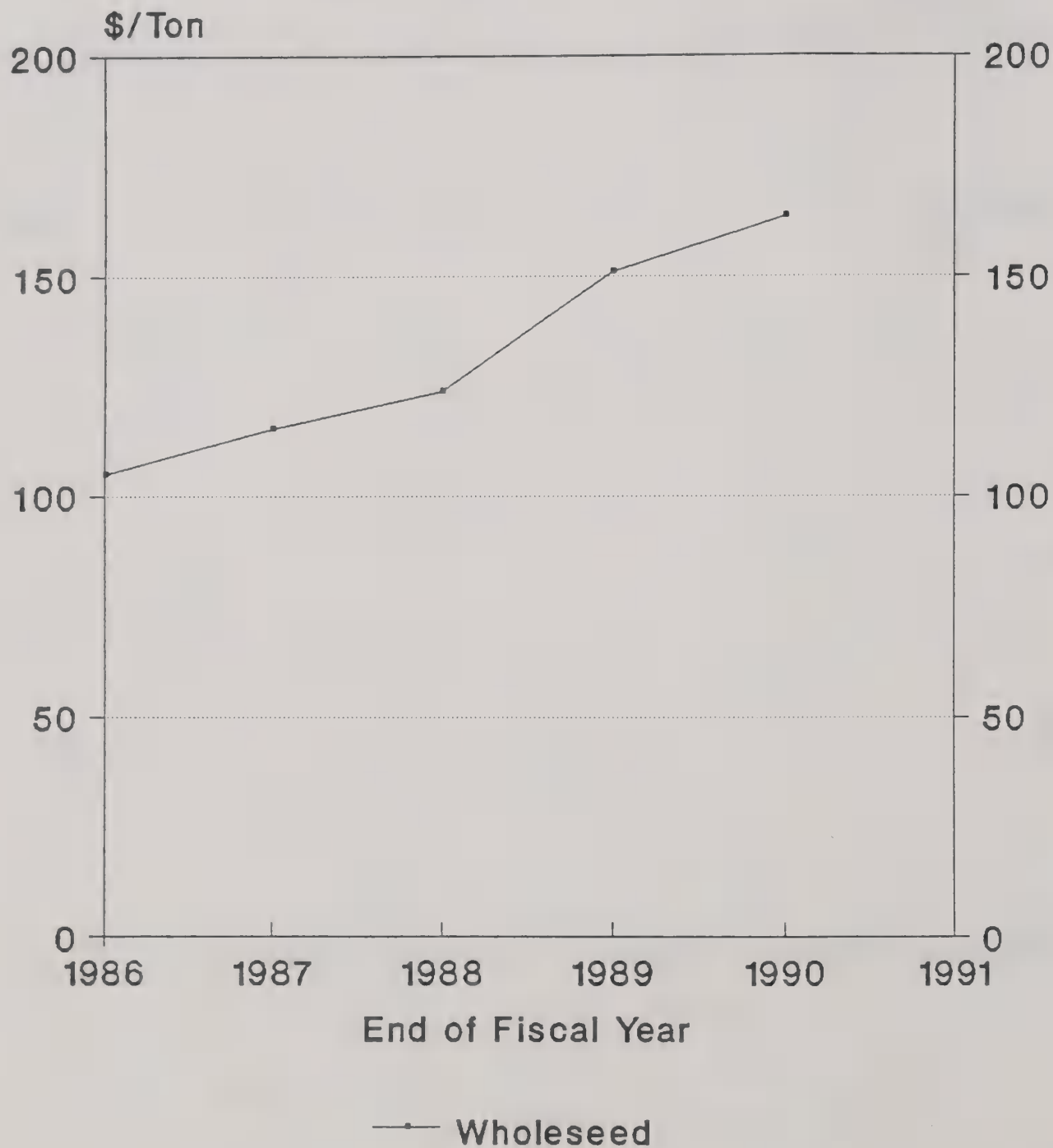


Table 9 -- Power utilization and cost, 1989-90

Oil mill	: 1,000 BTU : : per ton : : cottonseed :	KWH per : ton : cottonseed :	Cost (\$) : per : KWH	Cost (\$) : per ton : cottonseed :	Cost (\$) : per 1000 : BTU's
1	470	138	0.0605	8.34	0.0177
2	478	140	0.0385	5.39	0.0113
3	438	128	0.0312	4.00	0.0091
4	385	113	0.0535	6.04	0.0157
5	364	107	0.0429	4.58	0.0126
6	423	124	0.0709	8.79	0.0208
7	439	129	0.0595	7.66	0.0174
8	409	120	0.0439	5.26	0.0129
9	654	192	0.0571	10.94	0.0167
10	536	157	0.0530	8.32	0.0155
Average	460	135	\$0.0511	\$6.93	\$0.0150
Median	439	129	\$0.0533	\$6.85	\$0.0156

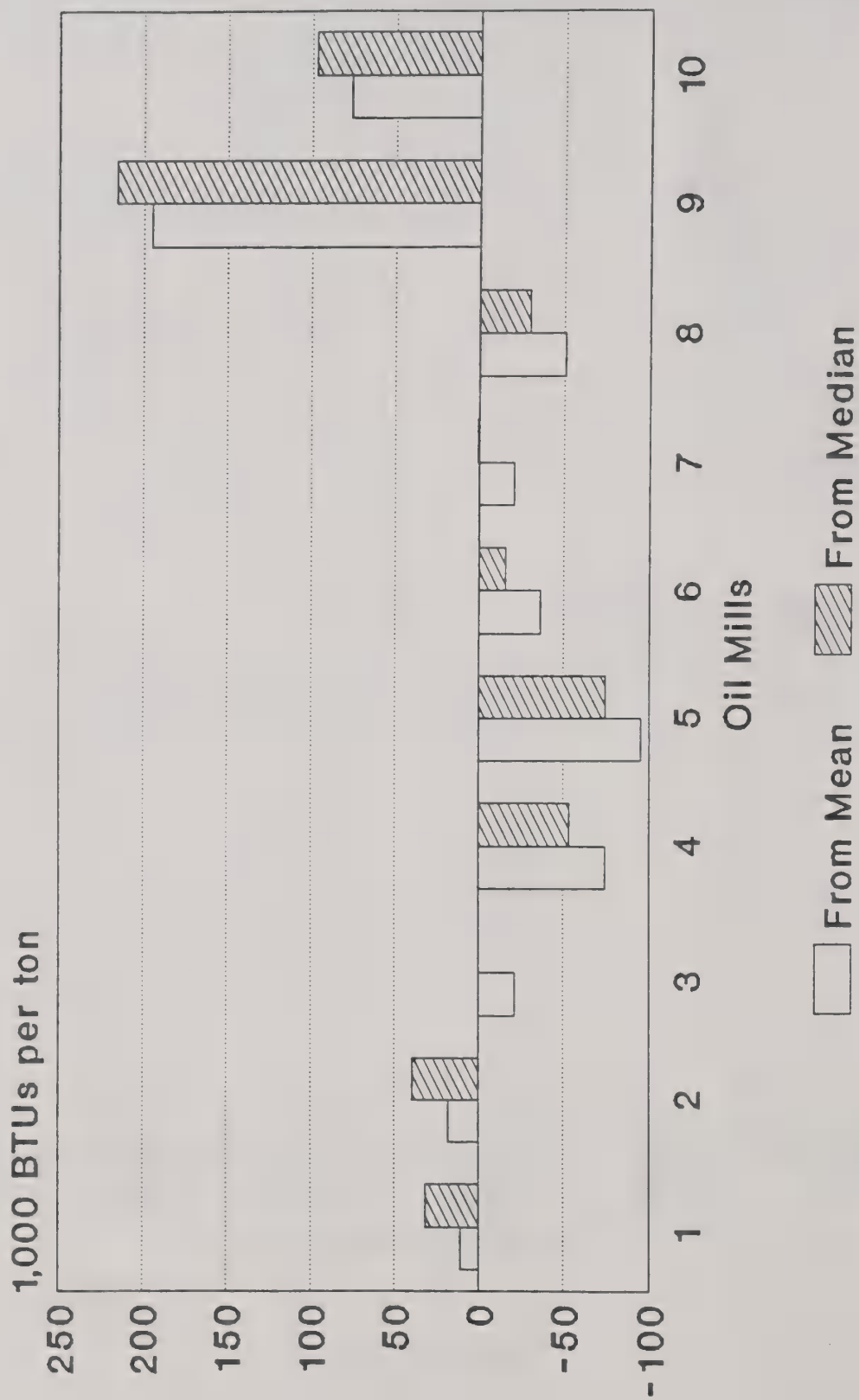
* Multiple plants.

Table 10 -- Power utilization in 1,000 BTU's per ton cottonseed, 1985/86-1989/90

Oil mill	1985-86	1986-87	1987-88	1988-89	1989-90
1	539	640	452	536	470
2	444	491	400	438	478
3	394	382	386	392	438
4	399	396	420	430	385
5	350	413	348	362	364
6	423	446	373	377	423
7	362	402	410	416	439
8	379	377	384	387	409
9	--	--	--	--	654
10	503	389	518	507	536
Average	358	366	231	462	460

* Multiple plants.

Fig. 15 - Power BTUs Differences
Differences from Mean and Median
1989-90



6 mills below mean

Table 11 --Fuel utilization and cost, 1989-90

Oil mill : 1,000 BTU : Fuel cost : Fuel cost: Fuel cost
 : per ton : (\$ per :(\$ per ton: (\$ per
 : cottonseed: m.c.f. : cottonseed: 1,000 BTU

1	1,280	2.91	3.61	0.0028
2	1,318	2.19	2.80	0.0021
3	1,150	3.19	3.56	0.0031
4	636	3.43	2.11	0.0033
5	970	2.24	2.11	0.0022
6	1,209	3.03	3.55	0.0029
7	1,151	2.50	2.79	0.0024
8	738	3.57	2.56	0.0035
9	870	3.22	2.71	0.0031
10	1,300	3.60	4.54	0.0035
Average	1,062	\$2.99	\$3.03	\$0.0029
Median	1,150	\$3.11	\$2.79	\$0.0030

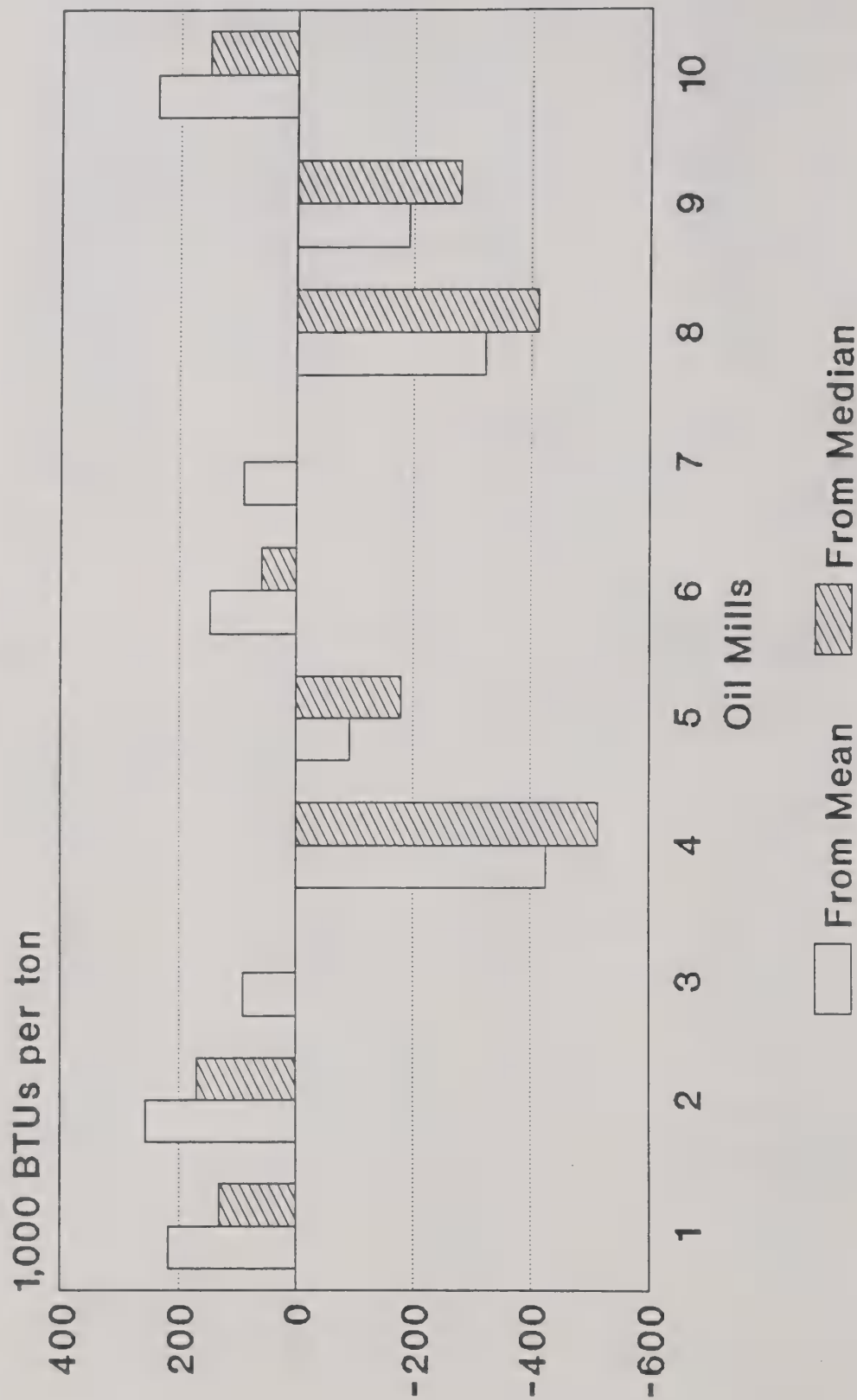
* Multiple plants.

Table 12 --Fuel utilization in 1,000 BTU's per ton cottonseed, 1985/86-1989/90

Oil mill	1985-86	1986-87	1987-88	1988-89	1989-90
1	1,438	986	985	1,055	1,280
2	1,216	1,336	1,196	1,195	1,318
3	1,017	1,017	982	934	1,150
4	701	701	753	711	636
5	907	1,201	947	920	970
6	1,123	1,186	1,099	1,138	1,209
7	771	924	1,056	1,034	1,151
8	705	--	--	699	738
9	--	--	--	--	870
10	839	1,014	896	728	1,300
Average	969	1,046	989	935	1,062

* Multiple plants.

Fig. 16 - Fuel BTUs Differences
Differences from Mean and Median
1989-90



4 mills below mean

Table 13 --Total energy utilization and cost, 1989-90

	: 1,000 BTU	: Energy cost	: Energy cost
Oil mill	: per ton	: per ton	: per 1,000
	: cottonseed:	cottonseed	: BTU
1	1,750	11.95	0.0068
2	1,796	8.19	0.0046
3	1,589	7.56	0.0048
4	1,021	8.15	0.0080
5	1,334	6.69	0.0050
6	1,632	12.34	0.0076
7	1,590	10.45	0.0066
8	1,147	7.82	0.0068
9	1,524	13.65	0.0090
10	1,835	12.86	0.0070
Average	1,522	\$9.97	\$0.0179
Median	1,515	\$9.38	\$0.0186

* Multiple plants.

Table 14 --Total energy utilization in 1,000 BTU's per ton cottonseed, 1985/86-1989/90

Oil mill	1985-86	1986-87	1987-88	1988-89	1989-90
1	1,977	1,626	1,437	1,591	1,750
2	1,661	1,827	1,596	1,633	1,796
3	1,412	1,399	1,368	1,326	1,589
4	1,100	1,097	1,173	1,141	1,021
5	1,257	1,614	1,295	1,282	1,334
6	1,547	1,632	1,472	1,515	1,632
7	1,134	1,326	1,466	1,450	1,590
8	1,084	377	384	1,086	1,147
9	0	0	0	0	1,524
10	1,342	1,403	1,414	1,235	1,835
Average	1,327	1,412	1,221	1,397	1,522

* Multiple plants.

Fig. 17 - BTUs per Ton Cottonseed
1989-90

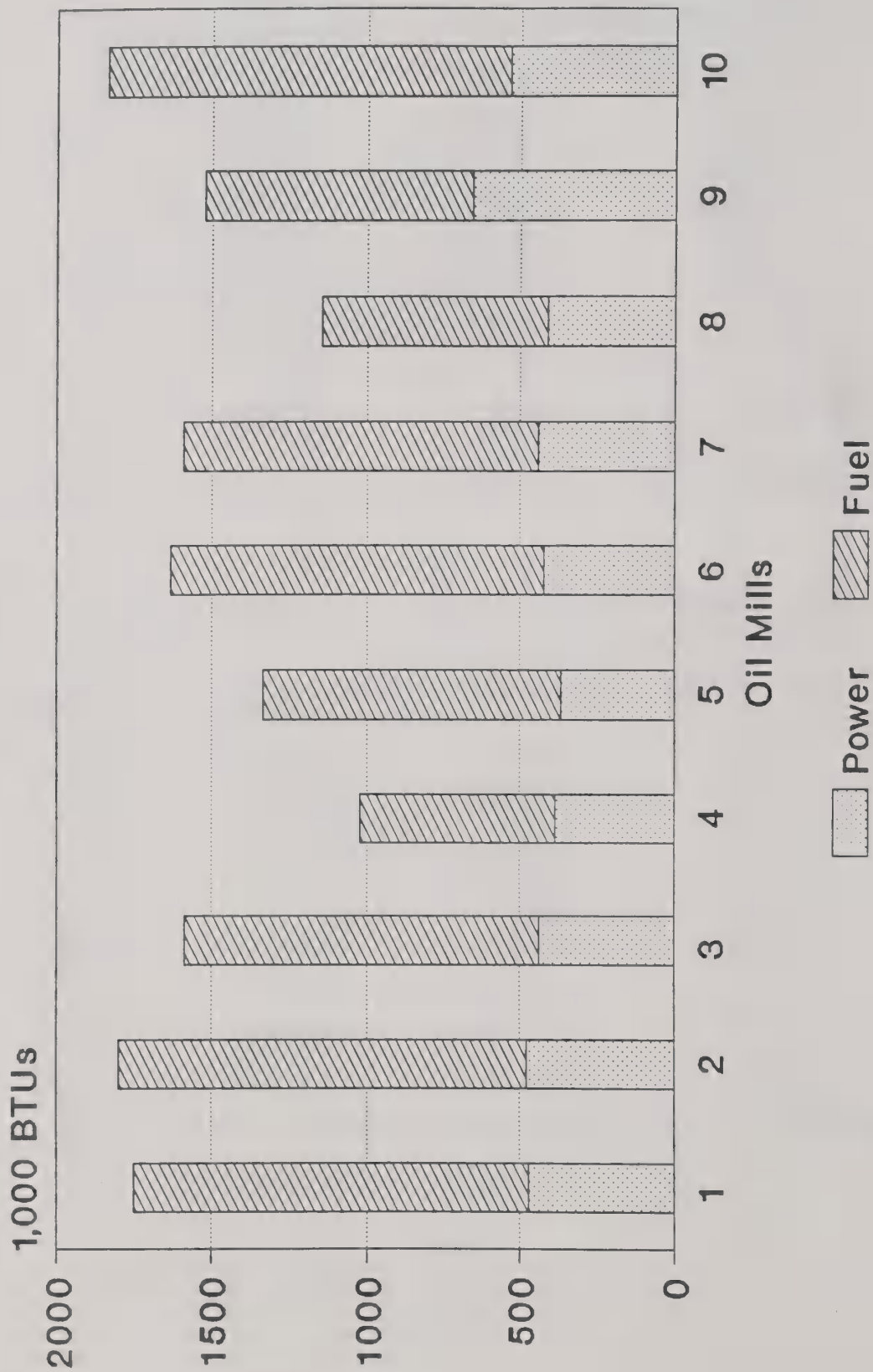


Table 15 --Labor utilization and cost per ton, 1989-90

Oil mill	: Labor cost : \$/ton	: Labor's cost : share (%) *	: Man hours : per ton
1	17.47	33%	1.90
2	18.19	40%	1.18
3	12.15	42%	1.55
4	12.52	35%	1.80
5***	9.38	30%	1.02
6	16.01	34%	1.52
7***	18.72	43%	2.28
8***	13.20	30%	1.58
9	17.59	35%	2.06
10	23.82	33%	2.70
Average	15.91	35%	1.76
Median	16.74	34%	1.69

* Share of manufacturing cost.

** Multiple mills.

*** Man hours data excludes sacking. Mill 5 sacked 10% of meal and 5% of hull sales. Mill 7 sacked 0.8% of meal sales and 31% of hull sales. Mill 8 sacked 6% of meal sales.

Table 16 -- Man hours per ton, 1985/86-1989/90

Oil mill	1985-86	1986-87	1987-88	1988-89	1989-90
1	1.52	1.82	1.39	1.32	1.90
2	1.40	1.74	1.00	1.03	1.18
3	1.73	1.79	1.56	1.66	1.55
4	1.61	1.33	1.31	1.36	1.80
5	1.24	1.32	0.91	0.82	1.02
6	2.44	2.08	1.94	1.39	1.52
7	2.01	2.10	2.27	2.32	2.28
8	1.15	1.01	1.16	1.18	1.58
9	--	--	--	--	2.06
10	3.30	3.08	2.65	2.60	2.70
Average	1.82	1.81	1.58	1.52	1.76

* Multiple plants.

Fig. 18 - Man Hours Differences
Differences from Mean and Median

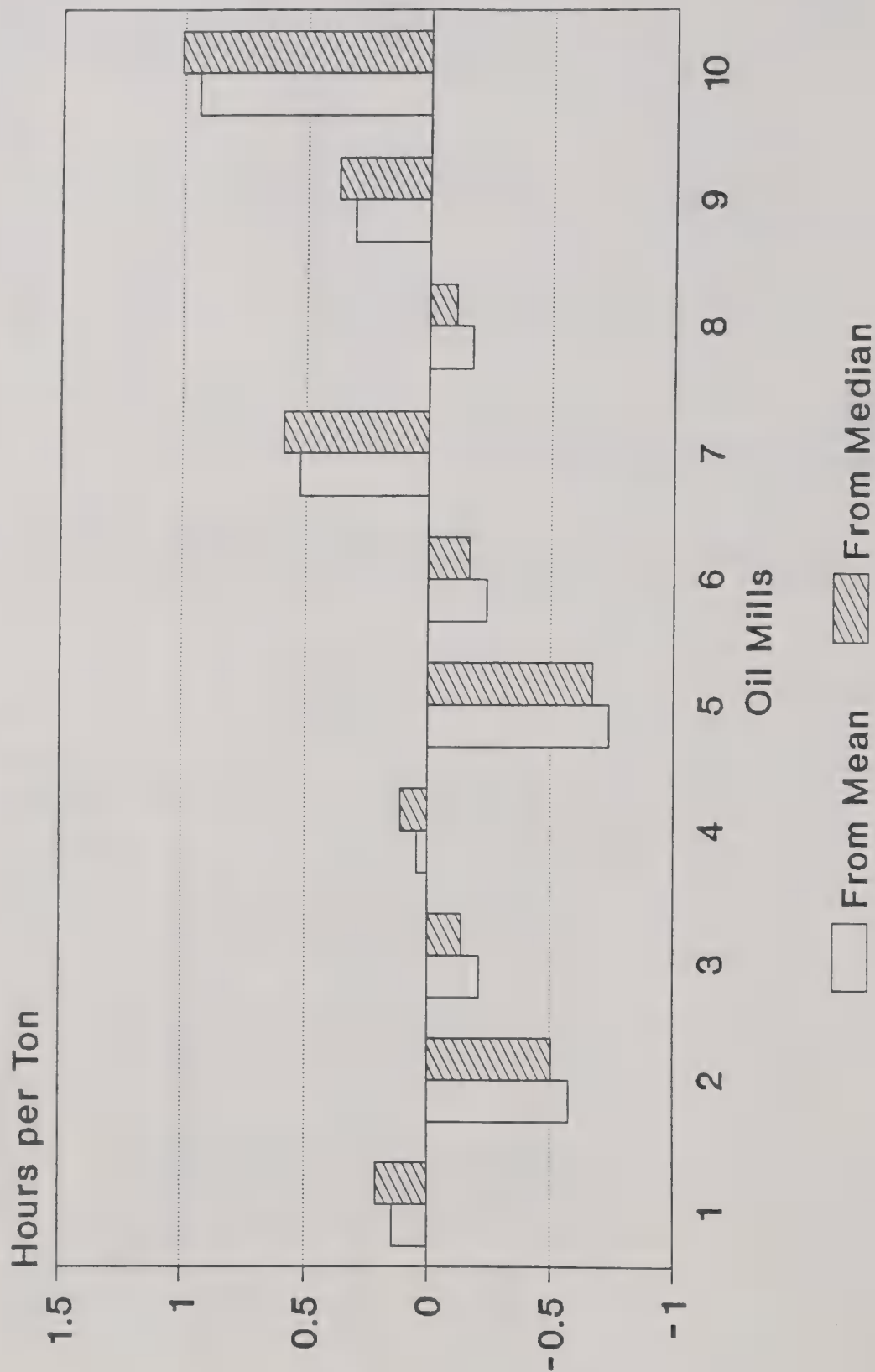


Table 17 --Solvent utilization and cost, 1989-90

Oil mill	: Cost per ton : cottonseed	: Gallons per ton : cottonseed
1	\$2.02	1.93
2	\$0.32	0.43
3	\$1.20	1.69
4	\$0.86	1.16
5	\$0.83	1.10
6	\$1.11	1.09
7	\$0.59	0.87
8	\$0.87	0.82
9	\$0.72	0.94
10	\$1.28	1.07
Average	\$0.98	1.11
Median	\$0.87	1.08

* Multiple plants.

Table 18 --Solvent loss per ton cottonseed,
1985/86-1989/90

Oil mill	1985-86	1986-87	1987-88	1988-89	1989-90
1	1.80	0.75	0.89	0.93	1.93
2	0.52	0.50	0.58	0.51	0.43
3	1.19	1.46	1.23	1.52	1.69
4	1.56	1.18	1.07	0.90	1.16
5	0.98	0.95	0.91	1.52	1.10
6	2.44	1.03	1.47	1.09	1.09
7	1.66	1.19	1.07	1.10	0.87
8	0.62	0.89	1.02	0.85	0.82
9	--	--	--	--	0.94
10	1.27	1.24	1.60	1.25	1.07
Average	1.34	1.02	1.09	1.07	1.11

* Multiple plants.

Fig. 19 - Solvent Loss Differences
Differences from Mean and Median
1989-90

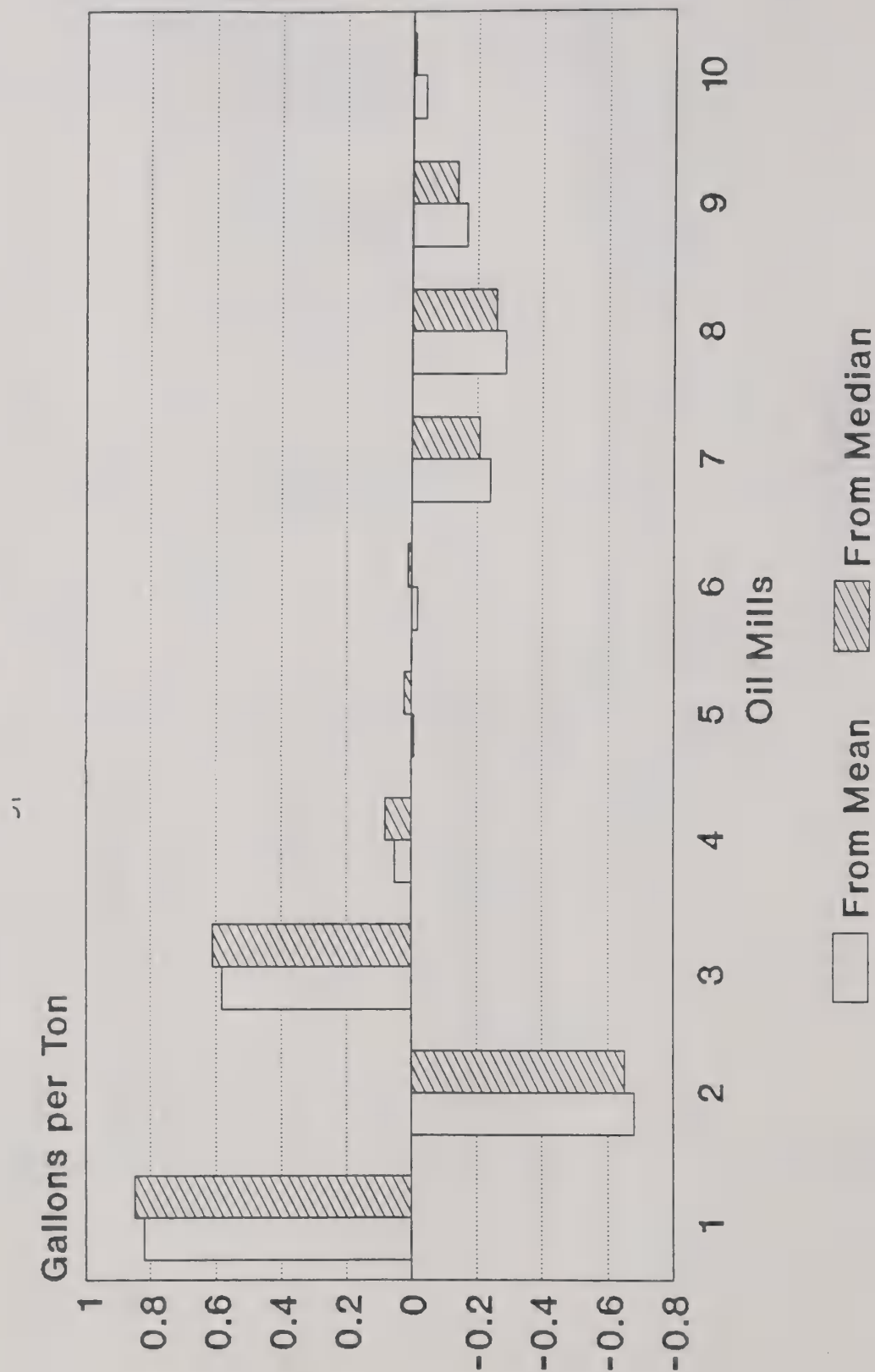


Table 19 - Separate averages for miscella and solvent oil mills,
1989-90

Item	5 Miscella Mills	4 Solvent Mills
Tons processed	492535	395282
Capacity utilization	54%	89%
Oil price (\$.01/lb)	24.64	21.08
Oil yield	313	344
Sales per ton:		
Oil	90.43	72.95
Meal	79.67	72.92
Other	55.29	52.56
Total	225.39	198.42
Cost per ton:		
Manufacturing	48.94	38.91
Other	24.16	20.03
Total	73.09	58.93
Net sales return	152.29	139.48
1,000 BTU per ton	1527	1458
Man hours per ton	1.71	1.79
Solvent loss per ton	0.87	1.20

*Key Components to Maximizing Yields
in the Crushing Industry*

John Wright
Owensboro Grain Company
Owensboro, KY



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

KEY COMPONENTS TO MAXIMIZING YIELDS
IN THE CRUSHING INDUSTRY

PRESENTED BY
JOHN M. WRIGHT
OF
OWENSBORO GRAIN COMPANY
FOR
47TH OILSEED CONFERENCE
NEW ORLEANS, LA
MARCH 8-10, 1998

Key Components to Maximizing Yields.

1. Proper Staffing and Training

- **Together**
- **Everyone**
- **Achieves**
- **More**

There is no letter “I” in the word “TEAM”

2. Accurate Measurement of Production

- **Allows Comparison to Industry Standards**

Performance Parameters Throughout

- 1. Flake Condition**
- 2. Dehulling Efficiencies**
- 3. Extraction Efficiencies**
- 4. Finished Product**
- 5. Equipment Maintenance**

4. AUTOMATION

Maximizing yields in the crushing operations is a critical measurement of how efficiently our operation is performing. And at Owensboro Grain Company, we use a multi-pronged approach to insure that our goals are met.

1. We believe that some perquisites must be established by the team before any achievable results can be realized.

We believe the following are key components to maximizing yields.

1. Proper Staffing and Training.
2. Accurate Measurement of Production.
3. Best Management Practices (BMP) Throughout the Process.
4. Automation.

PROPER STAFFING AND TRAINING.

a) Proper staff training is key. Is the staff up to the task? You need to assess your team's capabilities first before you can proceed with achieving your goals.

b) Once you have conducted a team assessment, and you are satisfied that your personnel can accomplish goals, you must then set goals. You must measure results by quantifying performances and you must reward excellence for achievement. We believe that rewarding goal achievement is necessary for continual success. We do this thru incentive systems based on performance. We have found that focusing on details and monitoring them yield the best results. We are able to do this with a team concept. After all - the word "TEAM" stands for:

Together
Everyone
Achieves
More

There is no letter "I" in the word "TEAM".

ACCURATE MEASUREMENT OF PRODUCTION

In order to set proper goals, it is important to accurately measure the quality and quantity of the raw material so that the outcomes will meet the expectations. For example, good measurement of oil content in the seed will allow for comparison of actual oil yields to industry standards. It is always good to be able to compare actual results to industry standards for comparison purposes.

BEST MANAGEMENT PRACTICES THROUGHOUT THE PROCESS

1. Flake Condition Can Improve Yields.

Flake conditions (i.e. thickness, moisture) play an integral part in oil yields. If there is not a good control over the quality of the flake to the extractor, then the effectiveness of extracting available free oil will diminish.

2. Dehulling Efficiencies.

Most crushing operations dehull 100% of their raw product, and removing hulls from the seed is not easy. Hull yields must be carefully watched; lower yields generally means a cleaner separation is made from the seed.

There are two benchmark parameters which we concentrate on to improve the yields, hull protein and hull oil.

3. Extraction Efficiencies.

To maximize yields in the extraction plant, three key parameters are monitored: white flake residuals, solvent loss, and energy consumption.

First consistently high extraction efficiencies will produce the best oil yield. Control of drainage, miscella concentration, vacuum, and temperature can produce these results for you.

Secondly, reducing solvent loss improves the yield from an efficiency standpoint. When plant operations are under control, solvent loss will drop thereby allowing plant yields to rise.

Energy consumption is the third area to improve yields in the extraction plant. We view yields as not only meaning product yield, but in-plant operations as well. The more efficient the plants are running, the better the operating yield will be as well. The less energy (steam, electricity) we expend, per unit of product, the more efficient our plants.

4. Finished Product.

Good measurement of product inventory is critical for accurate yield calculations. To maximize yields on finished product, a control system must be installed which maintains consistent moisture contents in the finished protein and hull products. If proper moisture contents are not maintained, yields will not be as great as they could be.

Trading rules allow the addition of flow enhancers to the finished product, up to 0.5% by weight. Adding these to the finished product will enhance the yields.

In our system, we keep a close eye on measured bulk density of the finished product. Unit weights play a key role in optimizing yields, and if the bulk

density measurements do not meet targeted goals, then yields will be adversely affected.

Finally, filling the grade on fiber content (hulls) will enhance yields.

5. Equipment Maintenance.

We believe that a very good maintenance program will enhance yields because it will improve process reliability and increase production as well. We think that good equipment maintenance, (preventative as well as predictive) improves the efficiencies of equipment, thus leading to better operational control.

AUTOMATIVE CONTROL OF OPERATIONS UTILIZING STATISTICAL PROCESS CONTROL

Automation has proven to be a key factor to enhanced efficiencies. The latest technological advances, when applied, have yielded improved efficiencies in processes. The ability to obtain real-time information and monitor plant operations improves the yields significantly. Automation will give better control, and better control nearly always means greater consistency. We have found over the years that better control means higher efficiencies; and the more efficient you are, the lower your physical losses become and the better the yields will be.

In conclusion, maximizing yields require a multi-pronged strategy to achieve. We have found that to start, you must have good people who are well-trained. We believe that, if you have good people, operating good equipment with accurate measuring tools, your task of trying to maximize your yields will be much easier to achieve.

Oil Refinery Yield Measurements

Giles Farmer

Applied Engineering & Science
Mabank, TX



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

*New Commodity Products from Soybean
Through Biotechnology*

Richard F. Wilson

Soybean & Nitrogen Fixation Research Unit
ARS, USDA
Raleigh, NC



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

New Commodity Products from Soybean Through Biotechnology

Richard F. Wilson, Soybean & Nitrogen Fixation Research Unit, Agricultural Research Service, United States Department of Agriculture, 4114 Williams Hall, 100 Derieux St., Raleigh, NC 27695-7620 U.S.A.

Research accomplishments in genetics, biochemistry and molecular biology are leading to fundamental changes in the seed composition of nearly every economically important oilseed crop. Some of these innovations, like mid-oleic sunflower, may replace the current commodity oil. Similar genetically-modified-oilseed (GMO) initiatives also may lead to new high-quality alternatives to other conventional agricultural commodities. As an example, biotechnological advances in soybean have produced a wide range of genetic resources needed to alter the concentration of several major and minor seed constituents. Considering market objectives and consumer demands, selected genetic traits are being combined to produce new commodity products that enhance soybean utilization. In that regard, the basic prototype composition for GMO soybeans likely will be: >44% protein, >20% oil, <3% 16:0, >60% 18:1 and <3% 18:3. Secondary priorities for modified oil composition include germplasm with high levels of saturated or polyunsaturated fat to improve specific edible and industrial applications. Hence, genetically modified soybean varieties will be defined by superior oil composition and protein quality. This discussion presents an overview of why these innovations are needed, how they were achieved, and targeted applications for these new commodity products.

Development of Low-Linolenic Acid Soybeans

ARS research has pioneered the emergence of genetically-modified-oilseed (GMO) technology in soybean. As GMO soybeans become established in the US soybean market, their impact on the oilseed industry will be measured against other GMO technologies such as: mid-oleic (18:1) sunflower and low-erucic (22:1), high-lauric (12:0) or low-linolenic (18:3) acid Canola. Successful GMO crops usually lead to the eventual replacement of a conventional commodity when the new technology has traits that improve consumer products and increase oilseed sales. Hence, the development of GMO technology rarely is serendipitous. There must be good reason, especially in the case of soybean which accounts for 85% of total US vegetable oil production.

The impetus to develop GMO soybeans with altered oil composition arose over a relatively long period from concerns in the oilseed processing industry about hydrogenation. Soybean oil needs to be hydrogenated for frying, baking or margarine applications. Unfortunately, hydrogenation gives rise to *trans*-isomers of unsaturated fatty acids in refined oil. Although this issue is subject to debate, new findings on *trans* fatty acids likely will bring some form of labeling requirement from FDA. Thus, products that require hydrogenated oils may become targets of consumer advocates. Recognizing the detrimental impact of such a scenario on our agricultural economy, the US soybean industry has taken steps to address this problem. One of the first actions was initiated over 20 years ago when the industry called for research to develop GMO soybean oils with low-18:3 concentration. However, that was before GMO technology had been invented. Therefore, this solicitation effectively marked the beginning of GMO technology for soybean.

Given what is known today, it may be hard to fully appreciate the initial impediments to the development of low-18:3 soybeans. At the time, there was no known genetic variation for any fatty acid among the 15,000 accessions in the entire USDA soybean germplasm collection. The biochemical pathway for linolenic acid synthesis was unknown, as well. Scientific peers even postulated it was impossible to change soybean oil composition through plant breeding. If a high-risk research problem ever required a creative solution, this had to be it. Nevertheless, two ARS soybean breeding programs, at W. Lafayette IN and Raleigh NC, took up this challenge. Two different breeding approaches were employed to create genetic diversity for 18:3. At Raleigh, a new breeding method designed to break genetic linkages through intensive gene recombination lead to discovery of a recessive allele affecting conversion of 18:1 to 18:2 (1,2). Use of a chemical mutagen at W. Lafayette lead to discovery of a recessive allele affecting the conversion of 18:2 to 18:3 (3). In addition, metabolic studies with this germplasm at Raleigh revealed that 18:1 esterified to phosphatidylcholine in the endoplasmic reticulum was the precursor of 18:2 and 18:3 (4). This was new knowledge in the 1970's. More recent evidence has confirmed these alleles encode the predominant ω -6 and an ω -3 desaturase activities in soybean seed.

When combined in the homozygous recessive state, these two alleles lowered 18:3 concentration from about 8% to less than 3% of crude soybean oil (Table 1). At Peoria, the late Tim Mounts demonstrated that oxidative stability and flavor characteristics of *trans*-free refined low-18:3 oil was equal or superior to hydrogenated soybean oil (5). Subsequently low-18:3 germplasm, N85-2176 (southern maturity) and C1640 (northern maturity), were released in the public domain for variety development.

Table 1. Low-Linolenic GMO Soybeans

Germplasm	Genotype	18:1	18:2	18:3	18:1-D	18:2-D
		<i>g * kg⁻¹ crude oil</i>			<i>%</i>	<i>%</i>
Normal	AABB	203	565	83	76.1	12.7
N78-2245	AaBB	515	308	44	40.5	12.6
C1640	Aabb	214	603	40	75.0	6.2
N85-2176	Aabb	497	338	29	42.5	7.8
LSD _{0.05}		163	144	9	18.6	3.2

18:1-D, ω -6 Desaturation; $(18:2+18:3)/(18:1+18:2+18:3)*100$

18:2-D, ω -3 Desaturation; $(18:3)/(18:2+18:3)*100$

AA/aa, alleles for ω -6 desaturase; BB/bb, alleles for ω -3 desaturase

Discovery of Genes Governing Expression of Other Fatty Acids in Soybean Oil

This story might have ended with low-18:3 soybean germplasm, but discovery research often produces unexpected events. As an example, long before saturated fat became a dietary health issue, plant populations used to develop low-18:3 soybeans spontaneously yielded the first genetic resources for low-palmitic (16:0) acid (6). Study of low-16:0 soybean germplasm revealed two different alleles, at the FAP₁ and FAP₃ gene loci, governed the trait (Table 2). These genes normally are homozygous dominant, giving about 12% 16:0 in conventional soybeans. However, when germplasm heterozygous for these two genes are mated, as in N79-2077-12 x C1726, the progeny exhibited transgressive segregation for 16:0 (7). In other words, hybrids

were produced that had both higher and lower 16:0 concentration than the original parents. Two germplasm lines representing the homozygous double recessive genotype, N94-2575 (southern maturity) and C1943 (northern maturity) were released in the public domain. These lines exhibited less than 4% 16:0 in crude oil. C1727 also was released which had about 17% 16:0 (8)

Table 2. Low-16:0 GMO Soybeans

Germplasm	Alleles	Fatty Acid	
		16:0	18:0
		% of crude oil	
N79-2077-12	<i>Fap1 fap3</i>	6.0	3.6
C1726	<i>fap1 Fap3</i>	8.1	3.4
N94-2575 or C1943	<i>Fap1 fap3</i>	3.7	2.9
Normal	<i>Fap1 Fap3</i>	11.5	4.1

Years later, the impact of these discoveries became fairly significant. Genetic resources needed to modify 16:0 concentration in soybean oil were in hand when saturated fat emerged as a dietary health issue. However, effective use of these genes in traditional or molecular genetic research required establishment of links between alleles associated with the trait in germplasm, a given gene and the product of that gene. This information was of fundamental importance to plant breeding programs. As one example, the time to breed low-16:0 lines could be reduced several years by screening segregating populations with a cDNA probe for the gene or antibodies to an enzyme encoded by the allele. However, associating alleles that govern 16:0 concentration with a single gene product was not obvious because there are eight enzymatic steps in the glycerolipid synthetic pathway that influence 16:0 concentration in soybean (Table 3).

Table 3. Enzyme Catalyzed Reactions Involved in 16:0 Metabolism

Enzyme	Reaction
3-keto-acyl-ACP synthetase III	16:0-ACP Synthesis
3-keto-acyl-ACP synthetase II	16:0-ACP to 18:0-ACP
18:0-ACP desaturase	18:0-ACP to 18:1-ACP
16:0-ACP thioesterase	16:0-ACP to 16:0-CoA
18:1-ACP thioesterase	18:1-ACP to 18:1-CoA
Glycerol-PO ₄ Acyltransferase	16:0-CoA on Glycerol-PO ₄
AGP Acyltransferase	16:0-CoA on Acylglycerol-3-PO ₄
Diacylglycerol Acyltransferase	16:0-CoA and 16:0-DG to TG

Briefly, metabolic evidence showed that 16:0 was made at the expense of 18:1, but not 18:0 (Figure 1). With other metabolic data, this indicated 16:0 concentration was determined by the activity of an enzyme that removes 16:0-ACP from the fatty acid synthetase before it is converted to 18:1-ACP. Thioesterases catalyze such a reaction. Northern-blots of mRNA from these genotypes with cDNA probes for acyl-ACP thioesterase genes confirmed this hypothesis. Thus, the low-16:0 alleles controlled expression of a 16:0-ACP thioesterase in these germplasm.

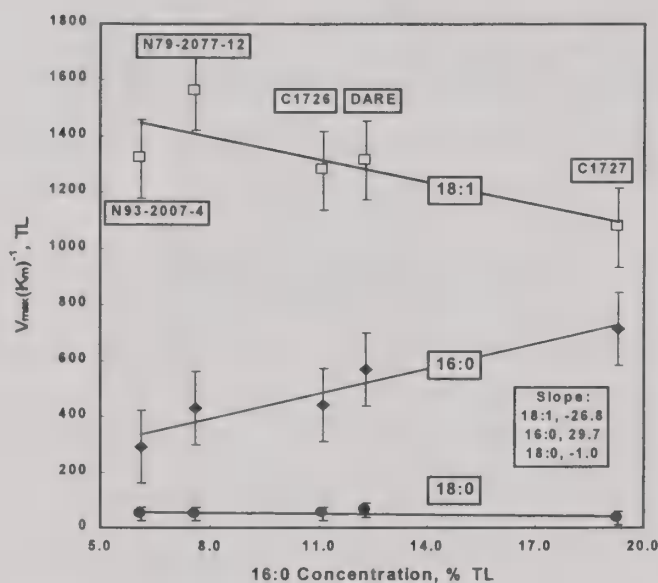


Figure 1. Effect of alleles governing 16:0 concentration on fatty acid synthesis in soybean germplasm. Genotypic differences in total fatty acid synthetic rates were determined via acetate saturation kinetics in developing seed at 35 days after flowering. First order rate constants were regressed against 16:0 concentration in total lipid.

The other major saturated fatty acid in soybean oil, stearic acid (18:0), is governed by a single gene locus. Metabolic evidence from germplasm having 3 to 36% 18:0 showed that 18:0 also was made essentially at the expense of 18:1, but not 16:0 (Figure 2). This could be attributed to differences in thioesterase activity. However, Northern-blot with a cDNA probe for the enzyme that converts 18:0-ACP to 18:1-ACP, suggest that 18:0 concentration in these germplasm primarily is determined by activity of the 18:0-ACP desaturase.

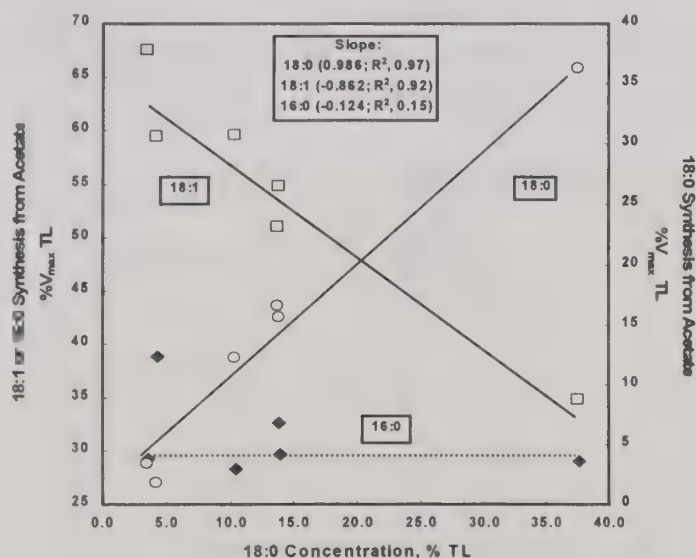


Figure 2. Effect of alleles governing 18:0 concentration on fatty acid synthesis in soybean. Genotypic differences in total fatty acid synthetic rates were determined via acetate saturation kinetics in developing seed at 35 days after flowering. The maximal velocity as a percentage of the rate of total fatty acid synthesis was regressed against 18:0 concentration in total lipid.

Understanding genetic regulation of fatty acid synthesis at this level has lead to an expansion of GMO technology in the public and private sector. In that regard, various approaches have been used to redirect lipid metabolism in soybean. Perhaps the most publicized example of GMO soybeans comes from DuPont, where the FAD2-1 gene was expressed in antisense orientation to restrict ω -6 desaturase activity (9). This alteration virtually shut down conversion of 18:1 to 18:2, and gave oil with almost 80% 18:1 (Table 4). Although this transgenic material was derived via gene manipulation at the molecular level, high-18:1 concentration also may be achieved through natural gene recombination and selection via plant breeding. As an example,

segregation of low-16:0 and low-18:3 alleles in a F₂ population gave genotypes ranging from 36 to 70% 18:1. Pure lines developed from this material will resemble mid-oleic sunflower oil.

Table 4. Development of high-18:1 Soybean Germplasm

Germplasm	16:0	18:0	18:1	18:2	18:3
F ₂ Population Range	% total lipid				
Low	7	3	36	17	2
High	10	6	70	48	3
Normal	10	4	22	56	8
DuPont (Transgenic)	9	3	79	3	6

Thus, what began as an attempt to determine the biochemical and genetic regulation of 18:3 has given a remarkable and totally unexpected array of genetic diversity for fatty acid concentration in soybean. Now, we have the ability to make genetic changes that not only effect any of the five major fatty acids in soybean oil, but also to merge these traits in nearly all possible combinations. This includes genetic resources for very low- or high-18:3; low or high 18:1; high 18:2 or 18:0, plus high or low-16:0. As another example, higher polyunsaturates were achieved by adding an alternate set of desaturase genes (10), discovered in wild soybean (*Glycine soja*), to cultivated soybean (*Glycine max*). Together, these genetic resources enable many GMO alternatives to conventional soybean oil. The ultimate potential of this technology has yet to be realized.

New Commodity Products from GMO Soybeans.

An action plan for commercializing GMO soybeans is being developed by the United Soybean Board (USB) through a prominent group of farmers, crushers and end-users. In addition, the vertically integrated venture by DuPont and Pioneer Hi-Bred International, *Optimum Quality Grains*, has helped quicken the pace for commercialization of GMO soybeans. The ARS role in these plans is to ensure GMO varieties are developed that have: the highest probability of impact on the major segments of the US vegetable oil market, yielding ability equal to or better than the best conventional cultivars, and the broadest possible production base. To that end, our group at Raleigh coordinates a USB sponsored collaboration among eight major public soybean breeding programs. This effort is producing GMO soybean varieties that can be grown from Minnesota to Mississippi and Missouri to Maryland; territory that encompasses all US soybean production.

Coordination of these research activities is critical to success. Release of too many GMO lines with different oil quality traits would lead to logistical problems in handling at crushing facilities. For that reason, we have set certain market oriented priorities for development of GMO soybeans with improved oil quality (Figure 3):

- Salad/Cooking oil accounts for 44% of the domestic market. Our number one priority is a GMO soybean oil that meets FDA requirements for a LOW-SAT label, and is stable without hydrogenation to limit *trans*-isomers. The only oil that currently meets these requirements is low-18:3 Canola. We plan to start releasing varieties with less than 3% 16:0, more than 60% 18:1, and less than 3% 18:3 by end of this year.

- Baking/frying/margarine oils account for 52% of domestic use. A high-saturate oil for these applications also should help reduce *trans*-isomers. Our target is 35% 16:0 + 18:0. Thus far we have increased total saturates to ca. 25%.
- Industrial or drying oils take up the rest of the domestic market. These oils have rather small market share, but great potential for growth. New oil based paints, ink-carriers and lubricants will be possible with GMO soybean oils having 85% polyunsaturates and/or epoxy acids. Germplasm exhibiting greater than 65% 18:2 plus 3% or 20% 18:3 are in development.

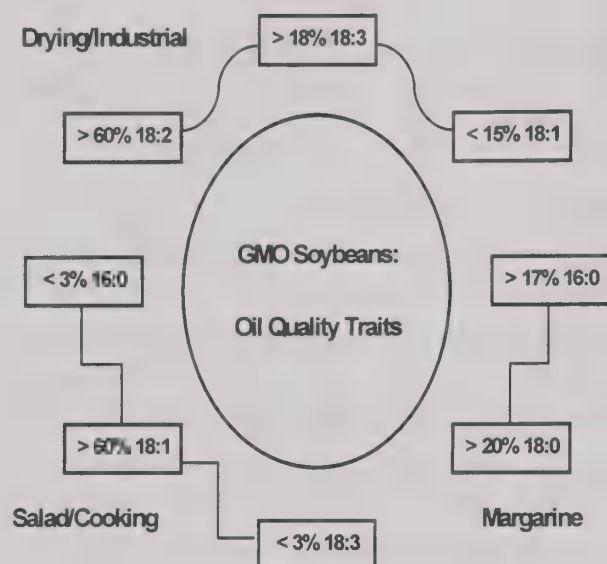


Figure 3. Primary trait combinations for GMO soybeans with improved oil quality.

Other traits also will be needed to enhance the value of GMO soybeans. For example, we have discovered that oil of low-18:3 genotypes has 50% more α -tocopherol plus a 2-fold greater concentration of stigmasterol than normal soybeans. These unexpected changes in high-valued minor seed constituents have apparent utility in manufacture of pharmaceutical products. The basis for the genetic association among these traits is under investigation. Research also has been initiated to increase the protein and oil content of GMO varieties (11). This objective is possible because ARS research at Raleigh discovered how to break the negative genetic relation between protein, oil and yielding ability. This means we can select for yield and maintain higher protein without sacrificing oil. That innovation guarantees production of 48% protein meal, and paves the way for production of a 50% protein meal or a high-fiber high-pro soy-meal for swine feed. It is not economically feasible to make the latter products today with conventional soybean varieties. Hence, developing the technology to achieve simultaneous genetic gain in protein, oil and yielding ability is another major breakthrough in soybean research. One that has immediate application and that will provide additional economic incentive to produce GMO soybeans with improved oil quality.

Summary

Redesigning the constituent composition of soybean in a manner that helps improve consumer product development and increases oilseed sales is a win-win situation for the US soybean industry. ARS research laid the foundation for, and continues to play a fundamental role in the

development of GMO technology in soybean. New market-oriented commodity products will issue from this work. Products that will help satisfy industry concerns, consumer demands, and enhance the competitive position of US soybeans in the world oilseed market. With time these new products may eventually replace conventional soybean oil. Given the example of Canola, and recent efforts to convert the sunflower industry to a mid-oleic oil, genetically modified soybeans are another important step toward new commodity products through biotechnology.

References

1. Burton, J.W., R.F. Wilson, C.A. Brim, and R.W. Rinne, Registration of Soybean Germplasm Lines with Modified Fatty Acid Composition of Seed Oil, *Crop Sci.* 29:1583 (1989).
2. Wilson, R.F., J.W. Burton and P. Kwanyuen, Effect of genetic modification of fatty acid composition of soybean on oil quality, in Proc. World Conf. Edible Oils and Fats Processing, edited by D. Erickson, Am. Oil Chem. Soc., Champaign, 1990, pp. 355-359.
3. Wilcox, J.R., and J.F. Cavins, Registration of C1640 Soybean Germplasm, *Crop Sci* 26:209-210 (1986).
4. Wilson, R.F., H.H. Weissinger, J.A. Buck and G.D. Faulkner, Involvement of phospholipids in polyunsaturated fatty acid synthesis in developing soybean cotyledons, *Plant Physiol.* 66:545-549 (1980).
5. Mounts, T.L., K. Warner, G.R. List, and R.F. Wilson, Low-linolenic acid soybean oils: Alternatives to cooking oils. *J. Am. Oil Chem. Soc.* 71:495-499. (1994).
6. Burton, J.W., R.F. Wilson, and C.A. Brim, Registration of N79-2077-12 and N87-2122-4, Two Soybean Germplasm Lines with Reduced Palmitic Acid in Seed Oil, *Crop Sci* 34:313 (1994).
7. Wilcox, J.R., J.W. Burton, G.R. Rebetzke, and R.F. Wilson, Transgressive segregation for palmitic acid in seed oil of soybean. *Crop Sci.* 34:1248:1250. (1994).
8. Erickson, E.A., J.R. Wilcox, and J.F. Cavins, Inheritance of altered palmitic acid percentage in two soybean mutants. *J. Heredity* 79:465-468. (1988).
9. Hitz, W.D., N. Yadav, R.S. Reiter, C.J. Mauvais and A.J. Kinney, Reducing polyunsaturation in oils of transgenic canola and soybean, in Plant Lipid Metabolism, edited by P. Mazliak, Acad. Press, The Netherlands, 1994, pp. 506-508.
10. Pantalone, V.R., G.J. Rebetzke, J.W. Burton, and R.F. Wilson. 1997. Genetic regulation of 18:3 concentration in *Glycine soja* accessions. *J. Am. Oil Chem. Soc.* 74:159-163.
11. Wilson, R.F. and J.W. Burton, Regulation of Linolenic Acid in Soybeans and Gene Transfer to High Yielding, High Protein Germplasm, in Proc. World Conf. Emerging Technologies in the Fats and Oils Industry, edited by A.R. Baldwin, American Oil Chemist's Society, Champaign, 1986, pp. 386-391.

*New Processes for the Conversion of Fats and Oils
to Higher Value-Added Products*

Thomas A. Foglia
ERRC/ARS/USDA
Wyndmoor, PA



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

NEW PROCESSES FOR THE CONVERSION OF FATS AND OILS TO HIGHER VALUE-ADDED PRODUCTS

Thomas A. Foglia*, George J. Piazza, An-Fei Hsu, R. D. Ashby, and William N. Marmer

**Eastern Regional Research Center, ARS, USDA
Wyndmoor, PA 19038**

Abstract

Fats and oils were once the primary sources of aliphatic carbon compounds used by industry. With the availability of inexpensive petroleum feedstocks, the consumption of these commodities has declined for most industrial applications. In addition, the economics have been exacerbated because of the large increase in world production of fats and oils without a similar increase in consumption. Moreover, health-related concerns continue to erode domestic and foreign market demand for edible fats and oils, especially animal fats. Despite their ready availability and competitive price the domestic non-food use of fats and oils continues to decline in almost all applications. To reverse these trends, our laboratory is evaluating the application of biocatalysis and biomimicry (chemical reactions that mimic enzyme reactions) to fats and oils with the goal of expanding current uses and identifying new uses of fats and oils in higher-value industrial applications. Particular areas of research in which we have developed expertise and continue to explore and develop include lipase reactions for fat and oil modification, biocatalytic oxygenation of fatty acids; biodiesel and biofuel additives, and biodegradable polymers.

Harvesting Erucic and γ -Linolenic Acids

The use of biocatalysts in transformations involving fats, oils, partial glycerides and fatty acids and their derivatives is well documented. One area where considerable effort is currently being expended is the study of the chemistry of lipases (triacylglycerol hydrolase, EC 3.1.1.3). More specifically, there has been a recent surge of interest in the application of lipases that exhibit either glycerol positional selectivity or fatty acid specificity. The expression of a lipase's selectivity for or against a given fatty acid structure can be exploited for the isolation of industrially or nutritionally important fatty acids from fats and oils (Figure 1). The expression of a lipase's selectivity is more pronounced in the esterification mode than in the hydrolysis mode. Using this selectivity concept, we recently developed a two-step process for obtaining highly enriched erucic acid fractions from high erucic acid rapeseed (HEAR) oil (1). Erucic acid was a targeted fatty acid because it already has several important industrial applications. The first step of the process was the total hydrolysis of HEAR oil using the lipase from *P. cepacia*. The latter lipase is a suitable catalyst for the total hydrolysis of triglycerides because the enzyme exhibits neither positional nor fatty acid selectivity. In the next step, the lipase of *G. candidum* was used to catalyze the esterification of the free fatty acids (FFA) of HEAR oil with 1-butanol. Because

the *G. candidum* lipase strongly discriminates against certain fatty acids, the erucic acid was concentrated in the FFA fraction. The results are summarized in Table 1. In a reaction conducted up to 52% conversion, the ester fraction contained 12.5% butyl erucate, and the residual FFA fraction contained 85.4% erucic acid. The HEAR oil used initially contained 47.5% erucic acid; therefore, the erucic acid content in the FFA fraction represented a total recovery of 86 % of the amount originally present in the oil. This two-step process also was used for the enrichment of γ -linolenic acid (GLA), an *n*-6-polyunsaturated fatty acid, in borage oil FFA (2). Borage oil is an excellent source of this nutritionally important fatty acid, as GLA comprises 25% of the fatty acids of this oil. The data in Table 1 show that in this manner one can obtain an acid fraction that contains >70% GLA with a total recovery of 95% of the GLA in the oil. Similar enrichments in GLA from primrose oil have been obtained with *M. miehei* lipase, though in lower absolute amounts because of the lower GLA (10%) content in the oil (3).

In the harvesting of a targeted fatty acid it is advantageous to use immobilized lipase preparations for practical considerations, such as enzyme reuse and ease of product isolation. To address this point, we used the supported lipases of *G. candidum* (4) and *M. miehei* (LipozymeTM) to obtain enriched GLA fractions. As shown in Table 1, the selectivities of both the supported lipases were equally effective in concentrating the GLA of borage oil FFA in esterification reactions. For both supported enzymes, GLA was recovered to the extent of about 80% in the FFA fraction. Additionally, the supported lipases could be recycled, and the recovery of GLA was about 70% after two additional reuses.

Biodegradable Polymers from Triglycerides

Poly(hydroxyalkanoates) (PHAs) are naturally occurring, optically active polyesters that accumulate in numerous bacteria as carbon and energy storage materials (5-7). In most cases the polymers contain β -linked repeat units and possess the general structure shown in Figure 2. The R group varies based on the bacterium and the carbon substrate from which the polymer was formed (8). Recently, there has been significant interest in the use of PHAs for biodegradable thermoplastics. Because they are viewed as "environmentally friendly," they are being studied as potential replacements for synthetic plastics in several applications. One major drawback to the use of these polymers is the cost involved in production. Generally, the cost to produce a given PHA polymer on an industrial scale is greater than for a comparable synthetic polymer. To make PHA production more economical, two avenues can be pursued: produce PHAs whose properties allow for their use in unique applications; lower the production costs either by increasing polymer yields or by using less expensive substrates. The latter possibility (and to some extent the former) can be achieved by using agricultural triglycerides as carbon substrates.

It is known that several bacteria (primarily pseudomonads) produce medium-chain-length PHAs from fatty acids (9-11). However, only recently have intact triglycerides been considered as feedstocks for PHA production. Three bacterial species have been shown to produce PHA from triglycerides. These include *Aeromonas caviae*, which produced a copolymer of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) from olive oil (12,13), *Pseudomonas aeruginosa*, which produced an complex PHA copolymer when grown on euphorbia or castor oil (14), and *Pseudomonas resinovorans*, which produced a PHA from tallow (11). In our laboratory we have

investigated the use of *P. resinovorans* to synthesize unique PHAs from other triglyceride substrates.

Six triglyceride substrates (lard, butter oil, olive oil, high oleic acid sunflower oil, coconut oil, and soybean oil) were screened as potential substrates for PHA production. A two stage fermentation was used to increase the number of viable cells prior to transfer into the polymer production medium in hopes that increased PHA yields could be achieved. Each triglyceride, whether animal fat or vegetable oil, supported cell growth (Table 2). This indicated that the organism showed no significant preference towards fats (solids), or oils (liquids) as substrates for growth and polymer production. After 48 h in the polymer production medium the cells were viewed under a phase-contrast microscope for the presence of phase-bright inclusions, evidence of polymer production. *P. resinovorans* produced an MCL-PHA from each triglyceride. This was evident by the presence of one or more PHA granules per bacterium that, when visually inspected, appeared to constitute approximately 50% of the cell mass. The cells were harvested by centrifugation and the cellular biomass, and PHA content and yield were determined (Table 2). The average PHA content for all tested triglycerides was 45%, and the average PHA yield was 1.5 ± 0.2 g/L. Thus our two stage fermentation system resulted in a 200% increase in PHA production compared to previously reported results (11). These results suggest that this system may be a viable means for commercial production of PHA polymers.

Catalytic Oxygenation of Fatty Acids to Reactive Intermediates

With one exception commercial fats and oils contain only double-bond and ester functionality, and for many non-food uses derivatization of a fat or oil to modify or increase its chemical functionality is required. The exception is castor oil, which contains the monohydroxyl fatty acid ricinoleic acid. The value that this hydroxyl imparts to castor oil is indicated by its market price, which is approximately three-fold higher than that of other vegetable oils. Another important industrial product is obtained by the epoxidation of vegetable oils. These derivatives are produced in excess of 200 million lbs per year in the US and are used mainly as stabilizer-plasticizers for PVC. Although the hydroxyl and epoxy functionalities are used in a number of applications, much of their value derives from their ability to be chemically transformed to other functional materials. Thus, for example, the hydroxyl group can be reacted to form a sulfate, endowing the fatty material with detergent properties, and the presence of the epoxide allows for easy crosslinking in plastics. Inexpensive ways of introducing oxygen into common fatty acids from US vegetable oils, e.g., soybean and cottonseed oils, in the form of hydroxy or epoxy functional groups has the potential to promote increasing utilization of these oils as industrial materials.

Our laboratory has an active research program designed to investigate novel methods for introducing oxygen into fats and oils. This research has revealed a number of promising avenues for the formation of oxygenated materials (Figure 3). We have previously reported our investigations on epoxidation of unsaturated fatty acids using the oxone method (15,16), and our use of the enzyme hydroperoxide lyase to produce intermediate length aldehydic materials (17). Here we will describe the results of our recent work to prepare fatty alcohol epoxides from polyunsaturated fatty acids.

The starting point for the synthesis is the preparation of a fatty acid hydroperoxide. This was accomplished using soybean lipoxygenase (LOX). This enzyme catalyzes the addition of oxygen to linoleic acid to form 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid (HPODE, Figure 3). LOX is a relatively unstable enzyme, and methods to immobilize this enzyme were sought in order to stabilize LOX and to allow for its recovery and reuse (18,19). Methods were devised to promote the formation of HPODE in organic solvents (20), and to promote the formation of hydroperoxide in esterified fatty acids such as those found in phospholipids and methyl esters (21).

After obtaining high yields of HPODE, methods of converting this material to useful chemical intermediates was sought. Any number of catalysts are capable of rearranging HPODE to alcohol epoxides, including strong acid and ferrous iron (22). However, what was desired was a catalyst that gives alcohol epoxy materials of specific structure, which limited the available catalysts to some enzymes and transition metal catalysts. A number of different metal catalysts were examined for their effect on the methyl ester of HPODE (Me-HPODE). The methyl ester was used because it was found that the free acid was non-reactive to these catalysts. It was also determined that very low water levels were required because water either inhibited the reaction or participated in the ring opening of the epoxide. In the rearrangement of Me-HPODE by titanium (IV) isopropoxide, the predominant methyl ester that was formed was the *threo* isomer, methyl 11(*R*),12(*R*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (23), whereas Nb(OC₂H₅)₅ gave its *erythro* analogue, demonstrating that both catalysts selectively promoted the formation of an α -epoxide. Thus these results demonstrate that the combination of a regio- and stereospecific LOX and a specific metal ion catalyst will produce a fatty alcohol epoxide of specific structure. This specificity can be used to design homogeneous industrial products.

Vegetable Oils and Fats for Renewable Fuels

There is an increasing interest in the development of alternative fuels to reduce the dependency of the United States on imported petroleum and to reduce the environmental burden from petroleum-based fuels. Considerable effort has been spent on the use of renewable fats and oils (triacylglycerols) as alternative diesel engine fuels since heavy-duty diesel engines are high emitters of pollutants (24-26). However, the high viscosity of vegetable oils in general has been recognized as one major impediment to their use as neat diesel fuels. Previous studies have shown that the viscosity of fats and oils can be reduced when they are converted to their respective monoalkyl esters (27-31). More recently, additional evidence has suggested other beneficial effects of monoalkyl esters in the form of lower emissions (32-34), improved biodegradability, and a no net contribution to the greenhouse effect when used as fuels known as biodiesel (33-34). In addition to biodiesel fuels, lubricants and lubricant additives also can be derived from fats and oils.

Tallow and soybean oil were chosen as the major material in our studies since the United States produces more tallow and soybean oil than the rest of the industrialized world (35). The demand for tallow in the global market has gradually decreased due to health concerns and competition from other fats and oils. Value-added products, such as nutraceuticals, cleaning

solvents, and biofuels, need to be developed from tallow to improve its commercial value. Recycled restaurant grease also was studied since it can be less expensive than tallow.

Previously, Nelson and coworkers (36) demonstrated the application of lipase-catalyzed transesterification to the production of alkyl esters - including methyl esters from soybean oil, rapeseed oil, tallow, and recycled restaurant grease - that could be used as biodiesel (Figure 4). Nelson and coworkers also showed that the low-temperature properties of monoalkyl esters derived from tallow and grease were significantly improved when branched alcohols were used for transesterification (37). Among the large varieties of alkyl esters synthesized, three alkyl esters, namely ethyl tallowate, isopropyl tallowate, and ethyl greasate, were selected for scale-up production and diesel engine performance tests, since preliminary data suggested their potential as biodiesel fuels (37). Physical and low-temperature properties of the three monoalkyl esters and their 20% blends in No. 2 diesel fuel are shown in Table 3. Properties of methyl soyate, the main form of alkyl esters currently available in the U.S. biodiesel market, also are included for comparison. Kinematic viscosities of the esters were close to the proposed ASTM specification for biodiesel (1.9 - 6.0 mm²/s) (38). Viscosity values for the ester-diesel blends (20:80; v/v) were in the acceptable range of 3.1 to 3.3 mm²/s, compared to 3.0 mm²/s for 20% methyl soyate-No. 2 diesel blend and 2.8 mm²/s for No. 2 diesel fuel. Viscosity of isopropyl tallowate (6.4 mm²/s) was higher than ethyl tallowate (5.2 mm²/s) due to the increased molecular weight of the isopropyl esters, which paralleled previous findings (37). In general, monoalkyl esters derived from grease seemed to have better low-temperature properties than the tallow esters (Table 3). The crystallization onset temperature (T_{co}) was determined because it can be used to predict cloud point (CP), which strongly correlates with cold-temperature properties of fuels.

The heating values of the three neat esters also are shown in Table 3. All three esters had approximately the same level of gross heat as methyl soyate, around 40,000 kJ/kg. These values are close to the reported heating value of No. 2 diesel fuel (Table 3). The ignition delay time of a fuel when injected into the combustion chamber of a diesel engine is indicated by its cetane number (39). Cetane numbers of the three neat esters were estimated using a spreadsheet calculation based on the weighted average cetane number for each individual fatty ester. The calculated cetane numbers were 65.9, 62.8, and 54.3 for ethyl tallowate, isopropyl tallowate, and ethyl greasate, respectively. The relatively high cetane numbers of the two tallow esters probably were from their high palmitic acid (16:0) content. Similarly, cetane number for ethyl greasate was closer to 50 since this ester contained less palmitate than the two tallowates and higher amounts of unsaturated fatty acids.

Results from the diesel engine performance and emissions tests for the ester-diesel blends are summarized in Table 4. By comparing performance of the two cylinders of the test diesel engine, we found that the ester-diesel blends resulted in a 1 to 3% higher indicated mean effective pressure (imep) than those from the diesel fuel and therefore carried 1 to 3% more of the load and provided 1 to 3% more power than the diesel fuel. The isopropyl tallowate- and ethyl greasate-diesel blends showed shorter injection durations and thus lower fuel consumption than the diesel fuel. These two fuel blends also had higher combustion efficiency than diesel fuel since they provided equal or better power output. On the other hand, the ethyl tallowate-diesel blend had longer injection durations and higher fuel consumption for essentially equal power output as the diesel fuel. Examination of selected areas of injector nozzles, cylinder heads, and piston faces after running the engine for 5 h showed that all three ester-diesel fuel blends

generated less carbon buildup than did the No. 2 diesel. Emissions for all three ester-diesel blend fuels were similar, with slightly lower CO₂ emissions and slightly greater O₂ emissions than those from the reference fuel, the No. 2 diesel fuel, while no apparent change in CO, HC or NO_x emission was found between the ester-diesel blends and the No. 2 diesel fuel. It has to be kept in mind, however, that engine durability and exhaust emissions data collected in this study are preliminary owing to the short duration of testing.

The composition of biodiesel has also received attention. Biodiesels were analyzed by capillary gas chromatography (40), which accounted for esters, triglycerides, diglycerides, and monoglycerides in one run. Another report included analysis of glycerol by GC (41). In the work described in both papers (40,41), the hydroxy groups of the glycerides and glycerol were derivatized by silylation with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide. Recently, a high performance liquid chromatographic (HPLC) method has been developed for quantifying reaction mixtures obtained from transesterified fats and oils (42). Advantages of the method are that derivatization of the sample is not required, analysis time is under 30 min and all neutral lipid classes, including alkyl esters, free fatty acids, triglycerides, 1,2- and 1,3-diglycerides and 1(2)-monoglycerides, are readily quantified.

This review therefore has shown the diverse applications of fats and oils when they are disassembled, reassembled, derivatized or even fed to bacteria. The United States produces enormous quantities of glycerides as coproducts of the oilseed processing, meat packing, and restaurant industries. Our research is giving industry the means to add value to these renewable commodities while giving the consumer new products that obviate the use of petroleum feedstocks.

References

1. Sonnet, P.E., T.A. Foglia, and S.H. Fearheller, Fatty Acid Selectivity of Lipases: Erucic Acid from Rapeseed Oil, *J. Am. Oil Chem. Soc.* 70:387-389 (1993).
2. Charton, E., and A. R. Macrae, Substrate Specificities of Lipase A and B from *Geotrichum candidum* CM/CC 335426, *Biochim. Biophys. Acta* 1123:59-64 (1992).
3. Syed Rahmatullah, M.K.S., V.K.S. Shukla, and K.D. Mukherjee, γ -Linolenic Acid Concentrates from Borage and Evening Primrose Oil Fatty Acids via Lipase-Catalyzed Esterification, *J. Am. Oil Chem. Soc.* 71:563-568 (1994).
4. Foglia, T.A., and P.E. Sonnet, Fatty Acid Selectivity of Lipases: γ -Linoleic Acid from Borage Oil, *J. Am. Oil Chem Soc.* 72:417-420 (1995).
5. Anderson, A.J., and E.A. Dawes, Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates, *Microbiol. Rev.* 54:450-472 (1990).
6. Brandl, H., R.A. Gross, R.W. Lenz, and R.C. Fuller, Plastics from Bacteria and for Bacteria: Poly(b-hydroxyalkanoates) as Natural, Biocompatible, and Biodegradable Polyesters, in *Advances in Biochemical Engineering/Biotechnology, vol 41*, edited by T.K. Ghose, A. Fiechter, Springer, Berlin, 1990, pp. 77-93.
7. Doi, Y., *Microbial. Polyesters*, VCH, New York, 1990.
8. Steinbuchel, A., H.E. Valentin, Diversity of Bacterial Polyhydroxyalkanoic Acids, *FEMS Microbiol. Lett.* 128:219-228 (1995).
9. Eggink, G., H. van der Wal, G.N.M. Huijberts, and P. de Waard, Oleic Acid as a Substrate for Poly-3-Hydroxyalkanoate Formation in *Alcaligenes Eutrophus* and *Pseudomonas Putida*, *Ind. Crops Products* 1:157-163 (1993).
10. Brandl, H., R.A. Gross, R.W. Lenz, and R.C. Fuller, *Pseudomonas Oleovorans* as a Source of Poly(b-hydroxyalkanoates) for Potential Applications as Biodegradable Polyesters, *Appl. Environ. Microbiol.* 54:1977-1982 (1988).
11. Cromwick, A.-M., T. Foglia, and R.W. Lenz, The Microbial Production of Poly(hydroxyalkanoates) from Tallow, *Appl Microbiol. Biotechnol.* 46:464-469 (1996).
12. Shimamura, E., K. Kasuya, G. Kobayashi, T. Shiotani, Y. Shima, and Y. Doi, Physical Properties and Biodegradability of Microbial Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), *Macromol.* 27:878-880 (1994).
13. Shiotani, T., and G. Kobayashi, Japanese patent application 93049, 1993.

14. Eggink, G., P. de Waard, and G.N.M. Huijberts, Formation of Novel Poly(hydroxyalkanoates) from Long-Chain Fatty Acids, *Can. J. Microbiol.* 41(suppl 1): 14-21 (1995).
15. Sonnet, P.E., and T.A. Foglia, Epoxidation of Natural Triglycerides with Ethylmethyldioxirane. *J. Am. Oil Chem. Soc.* 73:461-464 (1996).
16. Piazza, G. J., A. Nuñez, P. E. Sonnet, and T.A. Foglia, Two New Methods for Producing Epoxidized Oils for Industrial Uses, in *Proceedings of the 46th Oilseed Conference--Processing Efficiency: Meeting the Challenge*, American Oil Chemists' Society, Agricultural Research Service, National Cottonseed Products Association, Champaign, IL., 1997.
17. Nuñez, A., T.A. Foglia, and G.P. Piazza, Improved Method for Extraction of Hydroperoxide Lyase from *Chlorella*, *Biotech. Techniques* 9:613-616 (1995).
18. Hsu, A.-F., T.A. Foglia, and G.J. Piazza, Immobilization of Lipoxygenase in an Alginate-Silicate Sol-Gel Matrix: Formation of Fatty Acid Hydroperoxide, *Biotechnology Letters* 19:71-74 (1997).
19. Parra-Diaz, D., D.P. Brower, M.B. Medina, and G.J. Piazza, A Method for the Immobilization of Soybean Lipoxygenase, *Biotechnol. Appl. Biochem.* 18:359-367 (1993).
20. Piazza, G. J., D.P. Brower, and D. Parra-Diaz, Synthesis of Fatty Acid Hydroperoxide in the Presence of Organic Solvent Using Immobilized Lipoxygenase, *Biotechnol. Appl. Biochem.* 19:243-252 (1994).
21. Piazza, G.J., T.A. Foglia, and A. Nuñez, Soybean Lipoxygenase-Promoted Oxidation of Free and Esterified Linoleic acid in the Presence of Deoxycholate, *J. Am. Oil Chem. Soc.* 73:1045-1049 (1996).
22. Gardner, H.W., Oxygen Radical Chemistry of Polyunsaturated Fatty Acids, *Free Radical Biol. Med.* 7:65-86 (1989).
23. Piazza, G.J., T.A. Foglia, and A. Nuñez, Enantioselective Formation of an α,β -Epoxy Alcohol by Reaction of Methyl 13(*S*)-Hydroperoxy-9-(*Z*),11(*E*)-octadecadienoate with Titanium Isopropoxide, *J. Am. Oil Chem. Soc.* 74:1385-1390 (1997).
24. Mazed, M.A., J.D. Summers, and D.G. Batchelder, Peanut, Soybean and Cottonseed Oil as Diesel Fuels, *Transactions of ASAE* 28:1375-1377 (1985).
25. Samson, W.D., C.G. Vidrine, and W.D. Robbins, Chinese Tallow Seed as a Diesel Fuel Extender, *Ibid.* 28:1407 (1985).

26. Dunn, R.O. and M.O. Bagby, Low-Temperature Properties of Triglyceride-Based Diesel Fuels: Transesterified Methyl Ester and Petroleum Middle Distillate/Ester Blends, *J. Am. Oil Chem. Soc.* 72:895-904 (1995).
27. Bruwer, J.J., B.v.D. Boshoff, F.J.C. Hugo, J. Fuls, C. Hawkins, A.N. v.d. Walt, A. Engelbrecht, and L.M. du Plessis, In *Agricultural Energy*, Vol. 2, Biomass Energy/Crop Production, ASAE Publication 4-81, American Society of Agricultural Engineers, St. Joseph, MI, 1981, pp. 385-390.
28. Clark, S.J., L. Wagner, M.D. Schrock, and P.G. Piennaar, Methyl and Ethyl Soybean Esters as Renewable Fuels for Diesel, *J. Am. Oil Chem. Soc.* 61:1632-1637 (1984).
29. Natusch, D.F.S., D.W. Richardson, and R.J. Joyce, Methyl Esters of Tallow as Diesel Extender, *Proceedings, VI International Symposium on Alcohol Fuels Technology Conference*, 1-340-346, 21-25 May, 1984, Ottawa, Canada.
30. Richardson, D.W., R.J. Joyce, T.A. Lister, and D.F.S. Natusch, edited by W. Palz, J. Coombs, and D.O. Hall, Methyl Esters of Tallow as a Diesel Component, *Proceedings of the International Conference on Energy from Biomass*, 736-743 (1985).
31. Ali, Y., M.A. Hanna, and S.L. Cuppett, Fuel Properties of Tallow and Soybean Oil Esters, *J. Am. Oil Chem. Soc.* 72:1557-1564 (1995).
32. Ali, Y., M.A. Hanna, and L. I., Leviticus, Emissions and Power Characteristics of Diesel Engines on Methyl Soyate and Diesel Fuel Blends, *Bioresources Technology* 52:185-195 (1995).
33. Masjuki, H., A.M. Zaki, and S.M. Sapuan, A Rapid Test to Measure Performance, Emissions and Wear of a diesel engine fueled with Palm Oil Diesel, *J. Am. Oil Chem. Soc.* 70:1021-1025 (1993).
34. Sii, H.S., H. Masjuki, and A.M. Zaki, Dynamometer Evaluation and Engine Wear Characteristics of Palm Oil Diesel Emulsions, *Ibid.* 72:905-909 (1995).
35. Blanton, B., Market Report, *Render* 26:10-15 (1997).
36. Nelson, L.A., T.A. Foglia and W.N. Marmer, Lipase-Catalyzed Production of Biodiesel, *J. Am. Oil Chem. Soc.* 73:1191-1195 (1996).
37. Foglia, T.A., L.A. Nelson, R.O. Dunn, and W.N. Marmer, Low-Temperature Properties of Alkyl Esters of Tallow and Grease, *Ibid.* 74:951-955 (1997).

38. Howell, S., U.S. Biodiesel Standards - An Update of Current Activities, SAE Technical Paper Series 971687, In: *SP-1274, State of Alternative Fuel Technologies*, Society of Automotive Engineers, Inc., Warrendale, PA, 1997.
39. Owen, K. and T. Coley, *Automotive Fuels Reference Book*, 2nd ed., Society of Automotive Engineers, Inc., Warrendale, PA 1995.
40. Freedman, B., W.F. Kwolek, and E.H. Pryde. Quantitation in the Analysis of Transesterified Soybean Oil by Capillary Gas Chromatography. *J. Am. Oil Chem. Soc.* 63:1370-1375 (1986).
41. Plank, C., and E. Lorbeer. Simultaneous Determination of Glycerol and Mono-, Di- and Triglycerides in Vegetable Oil Methyl Esters by Capillary Gas Chromatography. *J. Chromatogr. A* 697:461-468 (1995).
42. Foglia, T.A. and K.C. Jones, Quantitation of Neutral Lipid mixtures Using High Performance Liquid Chromatography with Light Scattering Detection, *J. Liq. Chrom. Rel. Technol.* 20:1829-1838 (1997).

TABLE 1

Esterification of High Erucic Acid Rapeseed (HEAR) Oil and Borage Oil Fatty Acids by Lipases^a

Substrate	Lipase ^b	Conversion ^c	Acid ^d	Yield ^e
HEAR Oil	<i>G. candidum</i>	52E	12.5	86
		48A	85.4	
Borage Oil	<i>G. candidum</i>	67E	1.8	95
		33A	71.8	
	<i>G. candidum</i> /Si	64E	7.5	80
		36A	55.5	
	<i>M. miehei</i>	59E	8.4	78
		41A	47.2	

^aHEAR oil and borage oil free fatty acids esterified with 1-butanol in hexane (7, 10).

^b*G. candidum*/Si is *G. candidum* lipase supported on silica (6); *M. miehei* lipase is LipozymeTM.

^cConversion expressed as % acids esterified to butyl esters (E); % unreacted fatty acids (A).

^dwt% erucic acid or γ -linolenic acid in ester fraction and acid fraction, respectively.

^eTotal wt% recovery of erucic acid or γ -linolenic acid originally present in HEAR oil (47.5%) and borage oil (24.9%), respectively.

TABLE 2
Cell Dry Weights and Poly(hydroxyalkanoate) Polymer Content of *P. resinovorans* Grown on Triglyceride Substrates

Substrate	Cell Yield ^a (g/L)	PHA content ^b (% dry weight)	PHA yield ^c (g/L)
Control			
Oleic acid	3.8 (±0.3)	48.9 (±2.8)	1.9 (±0.2)
Animal fats			
Tallow	3.0 (±0.2)	39.8 (±2.0)	1.2 (±0.1)
Lard	3.6 (±0.3)	47.4 (±3.0)	1.7 (±0.2)
Butter oil	3.6 (±0.1)	47.0 (±2.3)	1.7 (±0.1)
Vegetable oils			
Olive	3.4 (±0.2)	43.1 (±2.2)	1.5 (±0.2)
Sunflower (high oleic)	3.1 (±0.2)	41.2 (±1.8)	1.3 (±0.2)
Coconut	3.8 (±0.3)	51.0 (±3.2)	1.9 (±0.2)
Soybean	2.9 (±0.2)	44.5 (±3.4)	1.3 (±0.2)
Averages (x) ^d	x = 3.3 (±0.2)	x = 44.9 (±2.6)	x = 1.5 (±0.2)

^aCell dry weight ± standard deviation (n = 3).

^bPHA per cell dry weight ± standard deviation (n = 3).

^cCalculated by multiplying the cell yield (g/L) by the PHA content (% dry weight) of the cells.

^dAverages do not include oleic acid values.

TABLE 3
Physical and Low-Temperature Properties of Alkyl Esters and Ester-Diesel Blends^{a,b}

Fuel	Viscosity (mm ² /s, 40°C)	LTFT (°C)	CFPP (°C)	CP (°C)	PP (°C)	T _{co} (°C)	Gross heat (kJ/kg)
Ethyl tallowate (ET)	5.2	13	12	15	3	17.8	39,623
Isopropyl tallowate (IPT)	6.4	19	5	9	3	10.6	40,268
Ethyl greasate (EG)	6.2	9	0	5	-1	9.4	39,984
Methyl soyate (MS)	4.3	2	-3	0	-2	-	39,800
ET/D (20:80 v/v blend)	3.1	-1	-10	-6	-12	3.7	-
IPT/D (20:80 v/v blend)	3.2	12	-8	-10	-19	-5.3	-
EG/D (20:80 v/v blend)	3.3	-3	-12	-12	-21	-4.7	-
MS/D (20:80 v/v blend)	3.0	-12	-14	-14	-21	-	-
No. 2 Diesel fuel (D)	2.8	-14	-27	-16	-23	-8.5	45,200

^aLTFT - low-temperature flow test; CFPP - cold filter plugging point; CP - cloud point; PP - pour point; T_{co} - crystallization onset temperature.

^bData taken from reference 37.

TABLE 4**Diesel Engine Performance and Emissions Tests for Ester-Diesel Blends (20:80, v/v)^a**

Test	Summary of observations (blends vs. diesel fuel)
Performance	0 to 2% advantage in load-carrying capacity; 1 to 3% higher indicated mean effective pressure (imep); shorter injection durations (EG/D and IPT/D blends)
Carbon buildup	All three ester-diesel blends showed modest improvement over diesel in buildup characteristics
Emissions	0.25 to 0.5 % reduction in CO ₂ ; less than 1% increase in O ₂ ; No apparent change in CO, HC or NO _x

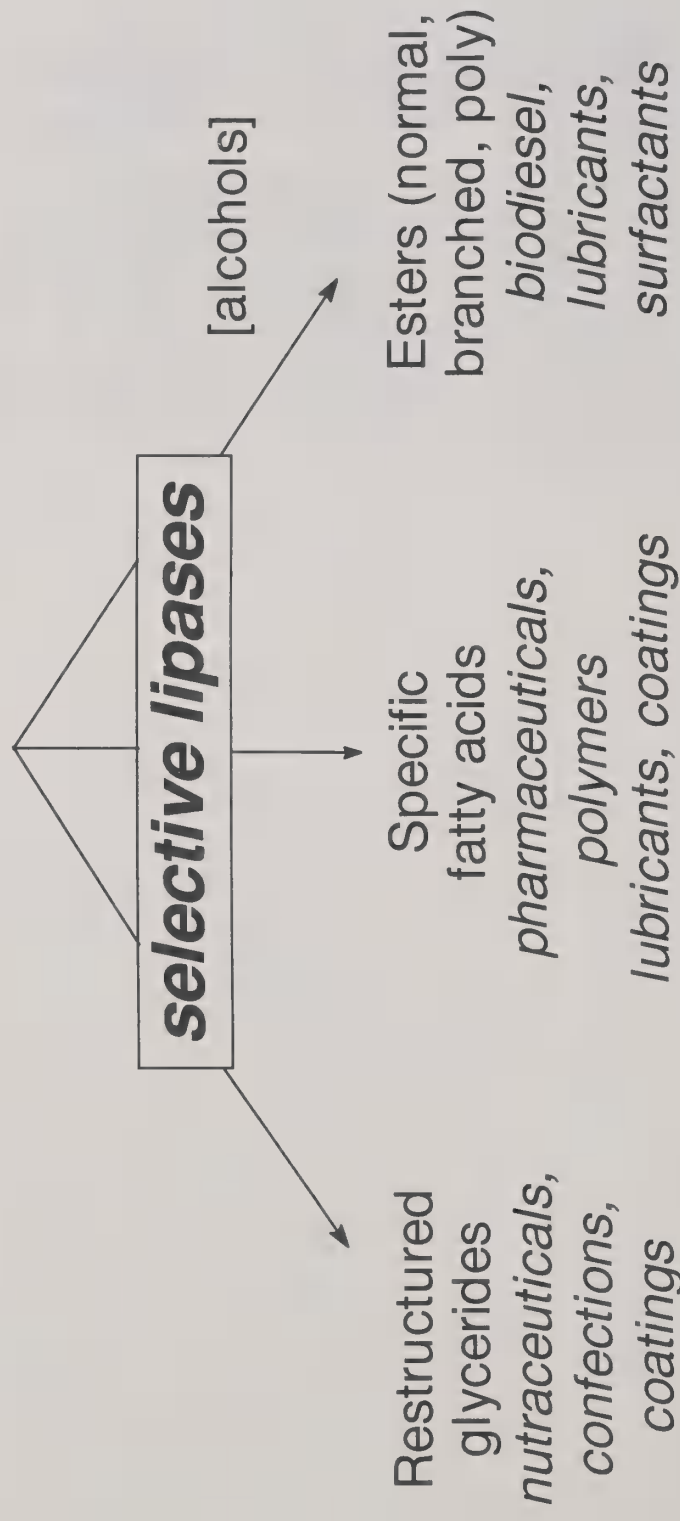
^aEster-diesel blends evaluated were ethyl, isopropyl tallowates and ethyl greasate.

Figure Legends

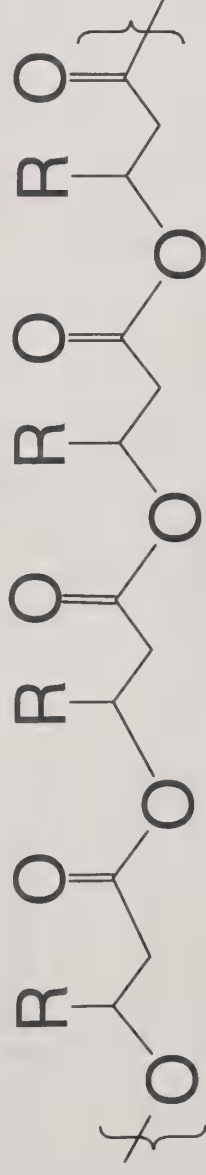
- Figure 1. Use of selective lipases to modify fats and oils to produce restructured glycerides, specific fatty acids and esters.
- Figure 2. The structure of short and long side-chain poly(hydroxyalkanoates) (PHA's). The long side-chain PHA's are produced from triglyceride feedstocks.
- Figure 3. Conversion of linoleate to oxygenated derivatives. The left side shows epoxide formation using the oxone method. The right side shows conversions using lipoxygenase to prepare the hydroperoxide. The hydroperoxide is then converted to alcohols, aldehydes, and epoxy alcohols.
- Figure 4. Characteristics of biodiesel produced using enzymes to prepare esters from tallow, greases, and soapstock.

FATS AND OILS

(tallow, lard, fish oils, vegoils, restaurant grease)



Poly(hydroxyalkanoates), PHA's



Short side-chain PHA's:

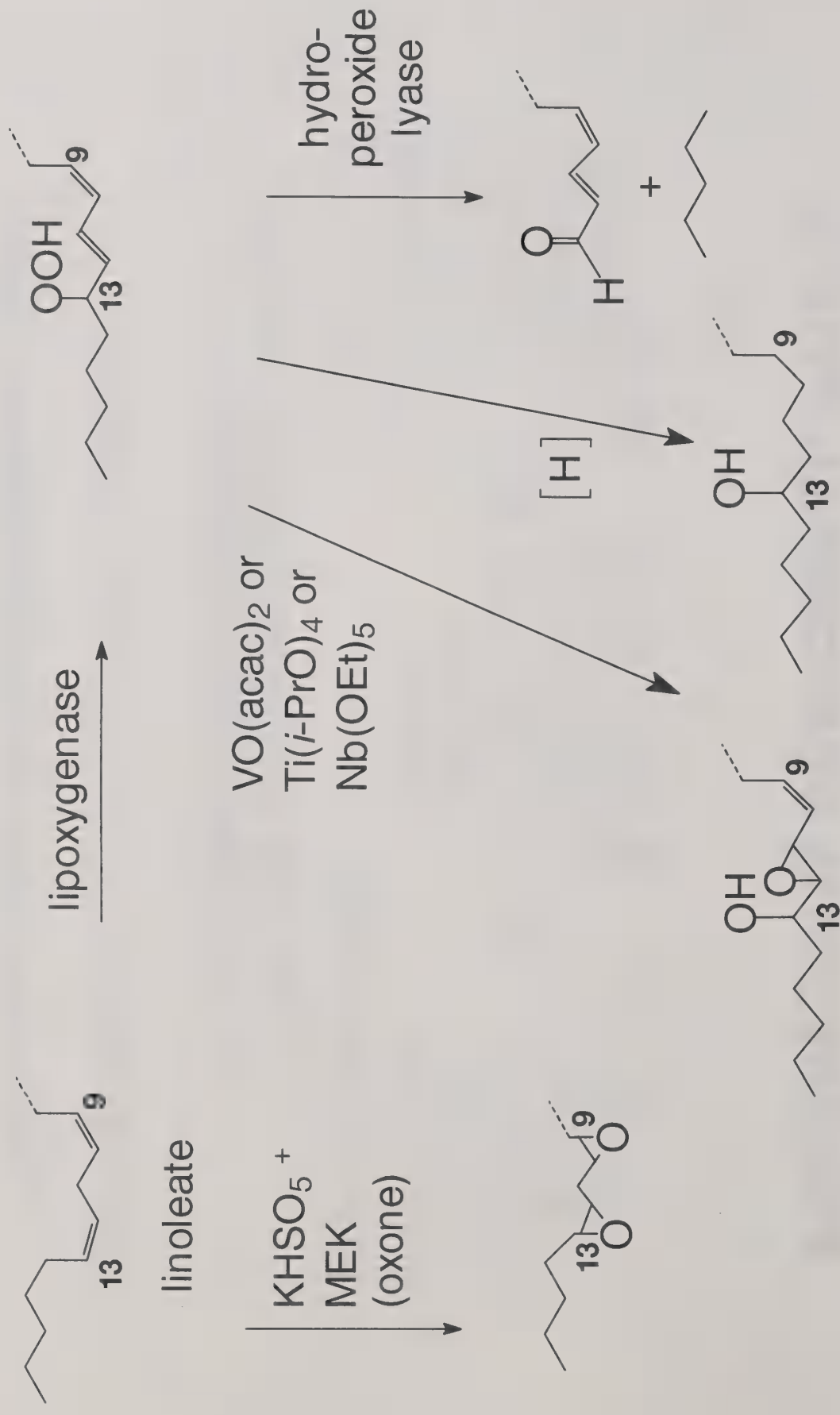
R = Me: PHB, poly(hydroxybutyrate)

R = Et: PHV, poly(hydroxyvalerate)

Long side-chain PHA's, from triglyceride feedstocks:

R = C₆₋₁₄ copolymers (some unsaturation)

Biocatalytic and biomimetic oxygenation



Biodiesel

- ❖ Enzymatic catalysis
- ❖ Inexpensive feedstocks
Tallow, Greases (High-FFA), Soapstock
- ❖ Branched alcohols (isopropanol, e.g.)
Normal alcohols (ethanol, e.g.)
- Adequate cold-temperature properties when blended 20:80 with petroleum diesel
- Tallow diesel: high cetane values, oxidative stability
- Grease: enzymes handle high FFA content

Oilseed and Oil Processing Research at ARS

Peter J. Wan
SRRC/ARS/USDA
New Orleans, LA



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Oilseed and Oil Processing Research at ARS

P. J. Wan
Southern Regional Research Center
Agricultural Research Service, USDA
New Orleans, Louisiana

Research in processing technology and chemistry of oilseeds and oils has always been one of the major emphasis of ARS. For more than 50 years, scientists and engineers of the ARS have contributed to the advancement of processing technology and better understanding of the oilseed and its oils. More recent examples are the alternative solvent to commercial hexane, the processing effect on available gossypol in cottonseed meal, free fatty acid determination of cottonseed, etc. The focus of our future research on oilseeds and their oils will be discussed.

The major research centers of the Agricultural Research Service, USDA were established to focus on the postharvest utilization research of agricultural commodities. Since early 1940's, much research resources have been invested in the development of processing technology and quality improvement of oilseeds and oils. Numerous well known scientists have contributed their talents in solving the technical problems associated with the oilseed and oil industries. A. E. Bailey spent five years at the Southern Regional Research Center from 1941 to 1946 and led a oil processing research section. During this period, Bailey finished his first edition of Bailey's Industrial Oil and Fat Products which immediately became the bible of the fats and oils industry. Besides this famous reference book, there are also more than 30 technical publications to his credit during his short tenure at the Center. His technical contribution covers a broad area. The following titles of selected publications will give you some idea of the nature of his work (1-9):

THE GRADING OF CRUDE VEGETABLE OILS BY MEANS OF REFINING TESTS - A REVIEW AND EVALUATION OF THE METHOD. (1942)

MODIFICATION OF VEGETABLE OILS. II. A COCOA BUTTER SUBSTITUTE FROM COTTONSEED OIL. (1943).

MEASUREMENT OF THE CONSISTENCY OF PLASTIC VEGETABLE FATS: A STANDARD MICROPENETRATION TECHNIQUE. (1944).

MOLECULARLY DISTILLED PEANUT OIL ANTIOXIDANTS AND PURE ALPHA TOCOPHEROL STABILIZING AGENTS FOR FATS OF POOR KEEPING QUALITY. (1944).

DILATOMETRIC INVESTIGATIONS OF FATS. I. APPARATUS AND TECHNIQUES FOR FAT DILATOMETRY. (1944).

THERMAL PROPERTIES OF FATS AND OILS. IV: SOME OBSERVATIONS ON THE POLYMORPHISM AND X-RAY DIFFRACTION CHARACTERISTICS OF TRISTEARIN AND A HIGHLY HYDROGENATED COTTONSEED OIL. (1945).

MODIFICATION OF VEGETABLE OILS. III. FRACTIONAL CRYSTALLIZATION OF FATTY ACIDS FROM SOLVENTS -- SEPARATION OF THE SOLID AND LIQUID ACIDS OF COTTONSEED OIL. (1945).

FLAVOR REVERSION IN EDIBLE FATS. (1946).

MODIFICATION OF VEGETABLE OILS. VI. THE PRACTICAL PREPARATION OF MONO- AND DIGLYCERIDES. (1946).

Most of the pioneer work described here have become commercial practices of the fats and oils industry. Another interesting subject related to the fat substitute, sucrose esters or Olestra the trade mark by Proctor and Gamble which was approved as a food additives in 1996, was also initiated at the Center in the 60's and 70's. Rubin Feuge and his colleagues at the Center contributed significantly on the esterification and purification technology for carbohydrate - fatty acid esters (10-28). Although the focus at the time was to develop a group of sucrose esters for wide varieties of formulation applications including confectioneries, emulsifiers, etc. After many decades of research in fat and oil technology, the research effort at the Center was allocated to high risk projects. One of the areas was to investigate alternative solvents to extract oil from oilseeds (29-40) and to remove and inactivate gossypol and aflatoxin (41-46). Ethanol is biorenewable and a desirable solvent for oilseed extraction. Its technical feasibility to extract oil and antinutritional components such as gossypol and aflatoxin from cottonseed has been demonstrated. However, the use of ethanol requires some modification of existing processing equipment. At the present time, the cost of using ethanol as extracting solvent still outweighs its technical benefits.

The environmental issues related to volatile organic compounds (VOC) and hazardous air pollutants (HAP) have once again been brought to the attention of commercial hexane users. Commercial hexane is regulated as a VOC which has a 100 tons emission loss limit per year for each plant. Even though commercial hexane has never been proven toxic, its main component, n-hexane, is a neural toxicant in animal study. Therefore hexane is also regulated as a HAP which has a lower regulated emission loss limit of 10 tons per year. At ozone non-attainable areas the limit of VOC will be less than 100 tons/year. Table 1 gives a set of calculated values of VOC which can help hexane users to determine the VOC emission at their extraction plants. Assuming that a plant is operating 250 days a year, with the known throughput tonnage per day and rate of solvent loss, gallon per ton of seed processed, one can quickly estimate the amount of VOC emission from his factory from this table. For instance, a 500 tons/day plant with solvent loss rate of half gallon per ton of seed processed, the plant will emit 172 tons of VOC in one year. This plant has to reduce the solvent loss rate to slightly less than 0.3 gallon per ton of seed processed to meet the current regulated VOC limit. Most soybean plants are processing greater than 1000 tons per day and are expected to exceed the VOC limit even at a solvent loss rate of 0.2 gallon/ton. For plants emitting more than 100 tons VOC per year, a federal operation permit is required and the solvent loss will be assessed at a fee set by the state government which is around \$40/ton. With the composition of commercial hexane, 55 - 85%, most of the soybean processors will also likely exceed the 10 tons/year HAP limit.

In an effort to search for a near term alternative to commercial hexane, we have demonstrated that commercial isohexane, which contains less than 2 % n-hexane, functioned quite well in both lab and plant trials (47-49). Commercial isohexane may not solve the VOC problem but it will allow most oilseed processing plants to operate below the HAP limit which can be seen from Table 2. The managers and operators of oilmills should also be reminded that commercial isohexane can also be a cost efficient solvent under most of the oilseed processing conditions. The take home message from this work is that isohexane will likely save energy and increase the throughput rate of an existing operation without major retrofit. The present research effort of evaluating the extraction performance of pure components of hexane and isohexane will help the producers and end users of commercial isohexane to formulate the most cost efficient mixtures of commercial isohexane. The findings will be reported at the upcoming annual meeting of American Oil Chemists' Society (AOCS) in Chicago this May.

In other research areas, we are establishing several analytical methods for the fats and oilseed industry. Using HPLC as a means to analyze gossypol in cottonseed products should be finalized in a year's time (50). Automated color measurements of refined and refined-bleached-deodorized oils have been studied through an international collaborative effort. An official method has been derived from this effort and will be included in the new edition of AOCS Manual for Standard Methods (51-53). A quick method for the determination of free fatty acid in seed is especially useful to the cottonseed industry. Preliminary results are encouraging. A simple protocol will be issued soon after potential users are convinced and willing to adopt the method. As a general practice, oilseed processors have to either convert soapstock into acid oil as feed additive or add the soapstock back to the meal. Composition of soapstock has been studied (54,55). Hopefully, optional value-added uses of soapstock can be recommended in the near future.

Since 1993, a major portion of our research resources has been allocated to the study of processing effect of cottonseed on its Total and Free gossypol and biologically available gossypol. This effort has been generously supported by both Cotton Incorporated and National Cottonseed Products Association (NCPA) and the members of NCPA. This work appears to have a narrow target, but its effort is by no means little and its benefit could be far reaching. During this period, we have surveyed all the existing cottonseed operations which include expeller, pre-press solvent extraction, expander solvent extraction and direct solvent extraction mills. Results showed that expeller produced meals are consistently low in Free Gossypol and therefore low in available gossypol in livestock feeding results. On the other hand, the most common practiced expander extracted meals are generally high in Free Gossypol and their Free Gossypol contents are inconsistent and vary greatly. During efforts to develop a cost effective processing condition to produce consistently low Free Gossypol cottonseed meal, some proven inactivation methods have been verified. Ferrous sulfate again was demonstrated to be the most effective divalent salt. The effective level of ferrous sulfate appears to be less than 1% of the meal as previously recommended (56). The next objective for this research effort is to develop an efficient process to produce consistently low Free Gossypol meals.

Research in processing, utilization and characterization of vegetable protein rich products has always been a major thrust at the Center. A.M. Altschul led

the early effort between 40's and 60's. Liquid cyclone processed high protein cottonseed flour was first developed at the Center. Scientists at the Center were largely responsible for obtaining the FDA's approval of a low gossypol cottonseed flour as a food additive. Now the research of vegetable protein at the Center is aiming at the value added uses and innovative processing technology to isolate protein.

References

1. BAILEY, A. E.; FEUGE, R. O.; BICKFORD, W. G. THE GRADING OF CRUDE VEGETABLE OILS BY MEANS OF REFINING TESTS - A REVIEW AND EVALUATION OF THE METHOD. OIL SOAP 19 (6) 97-102 (1942).
2. KRAEMER, E. A.; SMITH, B. A.; BAILEY, A. E. MODIFICATION OF VEGETABLE OILS. II. A COCOA BUTTER SUBSTITUTE FROM COTTONSEED OIL. OIL SOAP 20 (11) 235-240 (1943).
3. FEUGE, R. O.; BAILEY, A. E. MEASUREMENT OF THE CONSISTENCY OF PLASTIC VEGETABLE FATS: A STANDARD MICROPENETRATION TECHNIQUE. OIL SOAP 21 (3) 78-84 (1944).
4. OLIVER, G. D.; SINGLETON, W. S.; BAILEY, A. E. MOLECULARLY DISTILLED PEANUT OIL ANTIOXIDANTS AND PURE ALPHATOCOPHEROL STABILIZING AGENTS FOR FATS OF POOR KEEPING QUALITY. OIL SOAP 21 (7) 188-193 (1944).
5. BAILEY, A. E.; KRAEMER, E. A. DILATOMETRIC INVESTIGATIONS OF FATS. I. APPARATUS AND TECHNIQUES FOR FAT DILATOMETRY. OIL SOAP 21 (9) 251-253 (1944).
6. BAILEY, A. E.; JEFFERSON, M. E.; KREEGER, F. B.; BAUER, S. T. THERMAL PROPERTIES OF FATS AND OILS. IV: SOME OBSERVATIONS ON THE POLYMORPHISM AND X-RAY DIFFRACTION CHARACTERISTICS OF TRISTEARIN AND A HIGHLY HYDROGENATED COTTONSEED OIL. OIL SOAP 22 (1) 10-13 (1945).
7. SINGLETON, W. S.; LAMBOU, M.; BAILEY, A. E. MODIFICATION OF VEGETABLE OILS. III. FRACTIONAL CRYSTALLIZATION OF FATTY ACIDS FROM SOLVENTS -- SEPARATION OF THE SOLID AND LIQUID ACIDS OF COTTONSEED OIL. OIL SOAP 22 (7) 168-174 (1945).
8. BAILEY, A. E. FLAVOR REVERSION IN EDIBLE FATS. OIL SOAP 23 (2) 55-58 (1946).
9. FEUGE, R. O.; BAILEY, A. E. MODIFICATION OF VEGETABLE OILS. VI. THE PRACTICAL PREPARATION OF MONO- AND DIGLYCERIDES. OIL SOAP 23 (8) 259-264 (1946).
10. FEUGE, R. O.; LANDMANN, W.; LOVEGREN, N. V. PROGRESS ON THE DEVELOPMENT OF CONFECTIONERY FATS. I: TEMPERING OF CONFECTIONERY FATS AND COATING COMPOSITIONS. CANDY IND TECHNOL 119 (2) 7-9 (1962).

11. FEUGE, R. O.; LANDMANN, W.; LOVEGREN, N. V. PROGRESS ON THE DEVELOPMENT OF CONFECTIONERY FATS. II: A NEW PROCEDURE FOR MAKING COCOA BUTTER-LIKE FATS. CANDY IND TECHNOL 119 (3) 15-16 (1962).
12. FEUGE, R. O. DERIVATIVES OF FATS FOR USE AS FOODS. J AM OIL CHEM SOC 39 (12) 521-527 (1962).
13. FEUGE, R. O. CONFECTIONERY FATS: THEIR CURRENT STATUS AND POTENTIAL MARKET. J AM OIL CHEM SOC 41 (4) 4,26,30,63 (1964).
14. FEUGE, R. O.; GAJEE, B. B.; LOVEGREN, N. V. NEW PROCESS OFFERS ECONOMICAL CANDY FATS. CANDY IND CONFECTION J 122 (13) 7,8,10,14 (1964).
15. FEUGE, R. O.; LOVEGREN, N. V.; GAJEE, B. B. PROCESS FOR THE PRODUCTION OF CONFECTIONERY FATS. U S PAT 3,431,116 MARCH 4, (1969).
16. FEUGE, R. O.; GAJEE, B. B.; LOVEGREN, N. V. COCOA BUTTER-LIKE FATS FROM FRACTIONATED COTTONSEED OIL. I: PREPARATION J AM OIL CHEM SOC 50 (2) 50-52 (1973).
17. LOVEGREN, N. V.; GAJEE, B. B.; GRAY, M. S.; FEUGE, R. O. COCOA BUTTER-LIKE FATS FROM FRACTIONATED COTTONSEED OIL. II: PROPERTIES. J AM OIL CHEM SOC 50 (2) 53-57 (1973).
18. GROS, A. T.; FEUGE, R. O. PROPERTIES OF THE FATTY ACID ESTERS OF AMYLOSE. J AM OIL CHEM SOC 39 (1) 19-24 (1962).
19. FEUGE, R. O.; WILlich, R. K. ESTERIFICATION PROCESS. U S PAT 3,119,849 JANUARY 28, (1964).
20. FEUGE, R. O.; ZERINGUE, H. J.; WEISS, T. J.; BROWN, M. PREPARATION OF SUCROSE ESTERS BY INTERESTERIFICATION. J AM OIL CHEM SOC 47 (2) 56-60 (1970).
21. WEISS, T. J.; BROWN, M.; ZERINGUE, H. J.; FEUGE, R. O. QUANTITATIVE ESTIMATION OF SUCROSE ESTERS OF PALMITIC ACID. J AM OIL CHEM SOC 48 (4) 145-148 (1971).
22. WEISS, T. J.; BROWN, M.; ZERINGUE, H. J.; FEUGE, R. O. INFLUENCE OF SOLVENT ON DEGREE OF ACYLATION IN THE FORMATION OF SUCROSE ESTERS. J AM OIL CHEM SOC 49 (9) 524-526 (1972).
23. FEUGE, R. O.; BROWN, M.; WHITE, J. L. SURFACE ACTIVITY OF GLYCEROL GLYCOSIDE PALMITATES. J AM OIL CHEM SOC 49 (11) 672-673 (1972).
24. ZERINGUE, H. J.; FEUGE, R. O. PURIFICATION OF SUCROSE ESTERS BY SELECTIVE ADSORPTION. J AM OIL CHEM SOC 53 (9) 567-571 (1976).
25. ZERINGUE, H. J.; FEUGE, R. O. PURIFICATION OF SUCROSE ESTERS BY ULTRAFILTRATION. J AM OIL CHEM SOC 53 (12) 719-721 (1976).
26. FISHER, G. S.; ZERINGUE, H. J.; FEUGE, R. O. SURFACE ACTIVITY OF PURIFIED SUCROSE PALMITATES. J AM OIL CHEM SOC 54 (2) 59-61 (1977).

27. FEUGE, R.D.; WHITE, J.L.; BROWN, M. PREPARATION OF FATTY ACID ESTERS OF POLYOL GLUCOSIDES. J AM OIL CHEM SOC 55 699-702 (1978).
28. FEUGE, R.O.; ZERINGUE, H.J., JR. METHOD OF PURIFYING FATTY ACID ESTER PRODUCTS. U S PAT 4,377,686 MAR 22 (1983).
29. KING, W. H.; FRAMPTON, V. L. PROPERTIES OF OIL EXTRACTED FROM COTTONSEED WITH ACETONE-HEXANE-WATER SOLVENT MIXTURE. J AM OIL CHEM SOC 38 (9) 497-499 (1961).
30. GOLDBLATT, L. A.; ROBERTSON, J. A. EXTRACTION OF AFLATOXIN FROM GROUNDNUT MEAL WITH ACETONE-HEXANE-WATER AZEOTROPE. INT BIODETERIOR BULL 1 (1) 41-42 (1965).
31. PONS, W. A.; EAVES, P. H. AQUEOUS ACETONE EXTRACTION OF COTTONSEED. J AM OIL CHEM SOC 44 (7) 460-464 (1967).
32. HRON, R.J., SR.; KUK, M.S. ACETONE EXTRACTED COTTONSEED MEALS WITHOUT CATTY ODORS. J FOOD SCI 54 (4) 1088-1089 (1989).
33. HRON, R.J.; KOLTUN, S.P. AN AQUEOUS ETHANOL EXTRACTION PROCESS FOR COTTONSEED OIL. J AM OIL CHEM SOC 61(9) 1457-1460 (1984).
34. ABRAHAM, G.; HRON, R.J.; KOLTUN, S.P. MODELING THE SOLVENT EXTRACTION OF OILSEEDS. J AM OIL CHEM SOC 65(1) 129-135 (1988).
35. KUK, M.S.; HRON, R.J., SR.; ABRAHAM, G. REVERSE OSMOSIS MEMBRANE CHARACTERISTICS FOR PARTITIONING TRIGLYCERIDE-SOLVENT MIXTURES. J AM OIL CHEM SOC 66 (9) 1374-1380 (1989).
36. CHAMPAGNE, E. T.; HRON, R. J., SR.; ABRAHAM, G. STABILIZING BROWN RICE PRODUCTS BY AQUEOUS ETHANOL EXTRACTION. CEREAL CHEM 68 (3) 267-271 (1991).
37. ABRAHAM, G.; DECOSSAS, K.M.; HRON, R.J.; KUK, M.S. PROCESS ENGINEERING EXONOMIC EVALUATION OF THE ETHANOL EXTRACTION OF COTTONSEED: PRELIMINARY ANALYSIS. J AM OIL CHEM SOC 68 (6) 418-421 (1991).
38. CHAMPAGNE, E.T.; HRON, R.J., SR. STABILIZING BROWN RICE TO LYPOLYTIC HYDROLYSIS BY ETHANOL VAPORS. CEREAL CHEM 69(2) 152-156 (1992).
39. CHAMPAGNE, E. T.; HRON, R. J. SR.; ABRAHAM, G. UTILIZING ETHANOL TO PRODUCE STABILIZED BROWN RICE PRODUCTS. J AM OIL CHEM SOC 69 (3) 205-208 (1992).
40. CHAMPAGNE, E.T.; HRON, R.J. STABILITY OF ETHANOL-EXTRACTED BROWN RICE TO HYDROLYTIC AND OXIDATIVE DETERIORATION. J FOOD SCI 57(2) 433-436 (1992).
41. GARDNER, H. K.; KOLTUN, S.P.; VIX, H. L. E. SOLVENT EXTRACTION OF AFLATOXINS FROM OILSEED MEALS. J AGRIC FOOD CHEM 16 (6) 990-993 (1968).
42. HRON, R.J.; ABRAHAM, G.; KUK, M.S.; FISHER, G.S. ACIDIC ETHANOL EXTRACTION OF COTTONSEED. J AM OIL CHEM SOC 69(9) 951-952 (1992).

43. KUK, M.S.; HRON, R.J.; ABRAHAM, G.; WAN, P.J. ADSORPTIVE REMOVAL OF AFLATOXINS. J AM OIL CHEM SOC 69(11) 1154-1156 (1992).
44. KUK, M.S.; HRON, R.J.; ABRAHAM, G. ADSORPTIVE GOSSYPOL REMOVAL. J AM OIL CHEM SOC 70 (2) 209-210 (1993).
45. HRON, R. J. SR.; KUK, M. S.; ABRAHAM, G.; WAN, P. J. ETHANOL EXTRACTION OF OIL, GOSSYPOL AND AFLATOXIN FROM COTTONSEED. J AM OIL CHEM SOC 71 (4) 417-421 (1994).
46. HRON, R.J., SR.; WAN, P.J.; KUK, M.S. ETHANOL VAPOR DEACTIVATION OF GOSSYPOL IN COTTONSEED MEAL. J AM OIL CHEM SOC 73(10) 1337-1339 (1996).
47. WAN, P.J.; PAKARINEN, D.R.; HRON, R.J., SR.; RICHARD, O.L.; CONKERTON, E.J. ALTERNATIVE HYDROCARBON SOLVENTS FOR COTTONSEED EXTRACTION. J AM OIL CHEM SOC 72(6) 653-659 (1995).
48. WAN, P.J.; HRON, R.J., SR.; DOWD, M.K.; KUK, M.S.; CONKERTON, E.J. ALTERNATIVE HYDROCARBON SOLVENTS FOR COTTONSEED EXTRACTION: PLANT TRIALS. J AM OIL CHEM SOC 72(6) 661-664 (1995).
49. CONKERTON, E.J.; WAN, P.J.; RICHARD, O.A. HEXANE AND HEPTANE AS EXTRACTION SOLVENTS FOR COTTONSEED: A LABORATORY-SCALE STUDY. J AM OIL CHEM SOC 72(8) 963-965 (1995).
50. HRON, R. J.; KUK, M. S.; ABRAHAM, G. DETERMINATION OF FREE AND TOTAL GOSSYPOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. J AM OIL CHEM SOC 67 (3) 182-187 (1990).
51. WAN, P.J.; PAKARINEN, D.R. COMPARISON OF VISUAL AND AUTOMATED COLORIMETER FOR REFINED AND BLEACHED COTTONSEED OILS. J AM OIL CHEM SOC 72(4) 455-458 (1995).
52. WAN, P.J.; PAKARINEN, D.R.; HRON, R.J., SR. MISCELLA REFINING TEST METHOD FOR THE DETERMINATION OF COTTONSEED OIL COLOR. J AM OIL CHEM SOC 73(6) 815-817 (1996).
53. WAN, P.J.; HURLEY, T.W.; GUY, J.D.; BERNER, D.L. COMPARISON OF VISUAL AND AUTOMATED COLORIMETERS - AN INTERNATIONAL COLLABORATIVE STUDY. J AM OIL CHEM SOC 74(6) 731-738 (1997).
54. JOHANSEN, S.L.; SIVASOTHY, A.; DOWD, M.K.; REILLY, P.J.; HAMMOND, E.G. LOW-MOLECULAR WEIGHT ORGANIC COMPOSITIONS OF ACID WATERS FROM VEGETABLE OIL SOAPSTOCKS. J AM OIL CHEM SOC 73(10) 1275-1286 (1996).
55. DOWD, M.K. COMPOSITIONAL CHARACTERIZATION OF COTTONSEED SOAPSTOCKS. J AM OIL CHEM SOC 73(10) 1287-1295 (1996).
56. PROCEEDINGS OF THE CONFERENCE ON INACTIVATION OF GOSSYPOL WITH MINERAL SALTS. April 4-5, 1966. New Orleans, Louisiana. National Cottonseed Products Association, Inc. Memphis. TN.

TABLE 1. Annual VOC emission, tons/year, at various solvent loss rates, gallon per ton of oilseed crushed (1 year = 250 days)

Throughput Tons/day	Tons/year	Solvent loss rate, gallon per ton of seed crushed									
		0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	1.00	1.20
50	12500	5	7	9	10	12	14	15	17	34	41
100	25000	10	14	17	21	24	28	31	34	69	83
200	50000	21	28	34	41	48	55	62	69	138	165
300	75000	31	41	52	62	72	83	93	103	206	248
400	100000	41	55	69	83	96	110	124	138	275	330
500	125000	52	69	86	103	120	138	155	172	344	413
600	150000	62	83	103	124	144	165	186	206	413	495
700	175000	72	96	120	144	168	193	217	241	481	578
800	200000	83	110	138	165	193	220	248	275	550	660
1000	250000	103	138	172	206	241	275	309	344	688	825
1250	312500	129	172	215	258	301	344	387	430	859	1031
1500	375000	155	206	258	309	361	413	464	516	1031	1238
1750	437500	180	241	301	361	421	481	541	602	1203	1444
2000	500000	206	275	344	413	481	550	619	688	1375	1650
2500	625000	258	344	430	516	602	688	773	859	1719	2063
3000	750000	309	413	516	619	722	825	928	1031	2063	2475
3500	875000	361	481	602	722	842	963	1083	1203	2406	2888
4000	1000000	413	550	688	825	963	1100	1238	1375	2750	3300
4500	1125000	464	619	773	928	1083	1238	1392	1547	3094	3713
5000	1250000	516	688	859	1031	1203	1375	1547	1719	3438	4125

TABLE 2. Annual HAP emission, tons/year, at various solvent loss rates, gallon per ton of oilseed crushed (1 year = 250 days, 2% n-hexane)

Tons/day	Tons/year	Solvent loss rate, gallon per ton of seed crushed										
		0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	1.00	1.20	
50	12500	0	0	0	0	0	0	0	0	1	1	
100	25000	0	0	0	0	0	1	1	1	1	2	
200	50000	0	1	1	1	1	1	1	1	3	3	
300	75000	1	1	1	1	1	2	2	2	4	5	
400	100000	1	1	1	2	2	2	2	3	6	7	
500	125000	1	1	2	2	2	3	3	3	7	8	
600	150000	1	2	2	2	3	3	4	4	8	10	
700	175000	1	2	2	3	3	4	4	5	10	12	
800	200000	2	2	3	3	4	4	5	6	11	13	
1000	250000	2	3	3	4	5	6	6	7	14	17	
1250	312500	3	3	4	5	6	7	8	9	17	21	
1500	375000	3	4	5	6	7	8	9	10	21	25	
1750	437500	4	5	6	7	8	10	11	12	24	29	
2000	500000	4	6	7	8	10	11	12	14	28	33	
2500	625000	5	7	9	10	12	14	15	17	34	41	
3000	750000	6	8	10	12	14	17	19	21	41	50	
3500	875000	7	10	12	14	17	19	22	24	48	58	
4000	1000000	8	11	14	17	19	22	25	28	55	66	
4500	1125000	9	12	15	19	22	25	28	31	62	74	
5000	1250000	10	14	17	21	24	28	31	34	69	83	

*Supercritical Fluid Technology in the Oil Processing
and Conversion Industry*

Jerry W. King
NCAUR/ARS/USDA
Peoria, IL



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Supercritical Fluid Technology in the Oil Processing and Conversion Industry

Jerry W. King, Food Quality & Safety Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service/USDA, 1815 N. University Street, Peoria, IL 61604 U.S.A.

In the last decade there has been considerable diversification in applying supercritical fluid technology for other purposes than commodity oilseed extraction. In this presentation we will emphasize developments in three distinct areas: (1) enrichment of high value oil-derived chemicals from seed feedstocks, (2) integrating supercritical fluid-based operations for "green" processing and synthesis of oleochemicals, and (3) the analytical use of supercritical technology for the routine monitoring of oilseed quality. We will cite several examples of enrichment schemes that have been studied at NCAUR, including the enrichment of tocopherols from soybean oil, isolation of phospholipid fractions from deoiled seed meals and fractionation of glyceride mixtures using a thermal gradient column operated under supercritical fluid conditions. The role of reaction chemistry in supercritical media will also be discussed, including enzyme-catalyzed transformations in SC-CO₂, carbon dioxide's role in glycerolysis, and the potential of hydrogenating oils in the presence of supercritical fluid media. Products that have been synthesized include fatty acid methyl esters (FAME), emulsifiers, and potential margarine basestocks. The potential for coupling super- and subcritical processing in various combinations will also be noted. Examples include glycerolysis reactions initiated in the presence of SC-CO₂ followed by packed column fractionation of the resultant glyceride mixture. Similarly, it has been shown that the hydrolysis of vegetable oils to produce fatty acid mixtures of industrial value is feasible under subcritical water conditions. Finally, the routine use of supercritical fluid extraction (SFE) for the determination of oil levels in seed moieties has become an official method of the AOCS/AOAC. Our role in facilitating the acceptance of this methodology will be described, including on-going studies using an enzyme-based reactions that permits the analysis of speciated fat components, according to the Nutritional Labeling & Education Act definition of fat or oil content.

Historical Perspective

The initial interface of supercritical fluid technology with the vegetable oil processing industry occurred in the early 1980's when the technology was examined as an alternative to conventional solvent extraction of commodity oils (Friedrich and List, 1982; Stahl, et al.,

Names are necessary to report factually on available data; however the USDA neither guarantees nor warrants the standard of the product, and use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

1988). Despite the promise of the new technology, the oilseeds industry was reluctant to incorporate this environmentally benign extraction of seed oils based on capitalization costs, the need for a truly continuous process that duplicated current liquid extraction technique, and historical reluctance to accept alternative processing technologies. Supercritical carbon dioxide-extracted oils were shown to be superior to liquid-extracted oils in color, gum content, and flavor properties; but also showed a greater propensity toward oxidation because of their depleted phospholipid content. Eggers (1985) however demonstrated that seed oils could be continuously deoiling at supercritical conditions, and to date, no definitive study has appeared which represents accurately the economics of the process (see Reverchon and Osseo, 1994).

Despite the reservations shown towards supercritical fluid extraction (SFE) by the commodity seed oil processing industry, there are cited examples of SFE being effectively applied for extracting speciality oils. An excellent tome which discussed the present status and breath of supercritical fluid technology in oil and lipid chemistry has been rendered by King and List (1996). Perhaps unappreciated by the oilseed industry are the other available options for using sub- and supercritical fluid processing for extracting, refining, reacting, and fractionating lipid-based materials. For example, List, et al. (1993) have shown that solvent-extracted vegetable oils can be effectively degummed and refined to a state that permits the processed oil to be directly subjected to deodorization after supercritical refining. This alternative approach to producing high quality vegetable oils removes the need for degumming after the extraction step, as well as the need for bleaching earths prior to the final deodorization step. This process is a continuous countercurrent refining process with the supercritical carbon dioxide (SC-CO₂) contacting the oil in a high pressure vessel containing Goodloe packing. Lecithin-enriched concentrates can be collected by venting out the bottom of the vessel. A schematic of the processing system is shown in Figure 1.

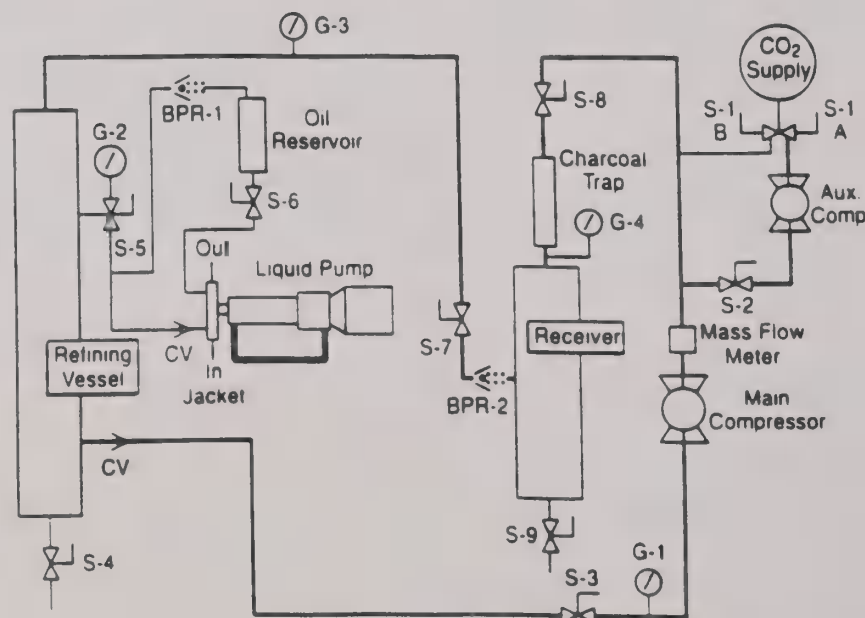


Fig. 1. Schematic of Continuous Supercritical CO₂ - Degumming Equipment. Legend: G, pressure gauge; BPR, back pressure regulator; CV, check valve; S, on/off valve.

In this paper, we shall show adaptability the supercritical fluid-based processing for the enrichment of high-value phytochemicals from seed oil feedstocks and for conducting reactions to convert seed oils to higher value oleochemical derivatives, as well as the exploitation of SC-CO₂ as an extraction/reaction medium in the analytical characterization of seed oils. These processes can be advantageously coupled together to offer some unique “green” processing opportunities as noted by King, et al. (1997a) and depicted in Figure 2. Here naturally-derived products can be altered by supercritical fluid-based processing, not only by SFE, but by coupling supercritical fluid fractionation (SFF), or supercritical fluid reaction (SFR) with the basic SFE isolation step, to produce a plethora of products or fractions.

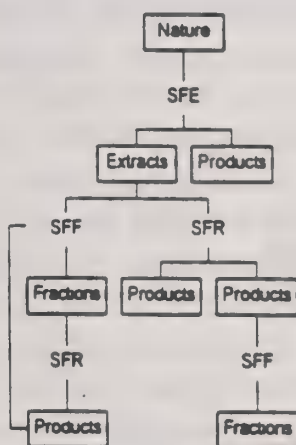


Fig. 2. Process Sequences Utilizing Supercritical Fluid Media for Isolating or Synthesizing Desired End Products

Supercritical fluid research at the National Center for Agricultural Utilization Research (NCAUR) involves a synergistic interaction between process development and analytical chemistry; often times yielding unexpected benefits across interdisciplinary lines. This will be illustrated using examples of phytochemical enrichment and enzyme-catalyzed reactions in both of the above-mentioned disciplines.

Oleochemical-Based Reaction Chemistry in Sub- and Supercritical Fluids

Supercritical fluid media have been receiving increased attention in reaction chemistry, since they can be substituted for conventional liquid solvents yielding a “green” synthetic process (Anastas and Williamson, 1996). Aside from their thermodynamic-based solvent properties, supercritical fluids offer enhanced conditions for favorable mass transport of reactants to catalyst surfaces, and can be coupled with the extraction mode to provide additional versatility to the oleochemical processor. These factors coupled with the potential acceleration of reaction rates at higher pressures and the ability to fractionate the synthesized products via pressure reduction, make supercritical fluids an attractive medium for reaction chemistry.

In some cases, SC-CO₂ can act as a reactant in a process or alternatively as a catalyst.

An example of the latter case is the effect of SC-CO₂ on conversion of vegetable oils to yield mixed glyceride compositions enriched in monoglyceride content. Temelli, et al. (1996) have shown that by subjecting a glycerolysis reaction to a pressure of 21 MPa at 250°C that mixtures of mono-, di- and triglycerides, equivalent to those obtained using alkali metal catalysts, can be synthesized. However absence of the metal catalysts in the SC-CO₂ initiated process eliminates the troublesome filtration step associated with the currently used industrial process. Further, the produced mixed glyceride compositions are much lighter in color than those synthesized using metal catalysts. These end products are the low cost end of the glyceride-based emulsifier market, and as will be shown later, can be enriched to a higher monoglyceride content via supercritical fluid fractionation.

Another area of activity at NCAUR is the use of enzyme-catalyzed conversion of lipids in the presence of SC-CO₂ /cosolvent mixtures. Our studies to date have centered on the use of Novozym SP 435 (Novo Nordisk, Franklinton, NC) as a multi-functional enzymatic catalyst for performing esterifications, acylations, transesterifications, glycerolysis and interesterifications (see Figure 3). Under appropriate conditions; temperatures less than 70°C and pressures in the interval of 17-41 MPa, the above conversions can be successfully achieved. Initial research by Jackson and King (1996) demonstrated that vegetable oils could be readily converted to their methyl esters after SFE of the seed moiety via transesterification over the supported Novozym SP 435 catalyst in the presence of flowing SC-CO₂ containing dissolved methanol. The synthesized methyl esters were identical in composition to those obtained by alkali metal initiated methanolysis. Again the advantage of the supercritical fluid synthesis becomes apparent since it eliminates catalyst filtration after completion of the reaction. The highly reproducible quantitative conversion of soybean and corn oils to their methyl esters via the enzymatic-catalyzed reaction in flowing SC-CO₂ suggested to us that the reaction might have some analytical utility which will be demonstrated later in the text.

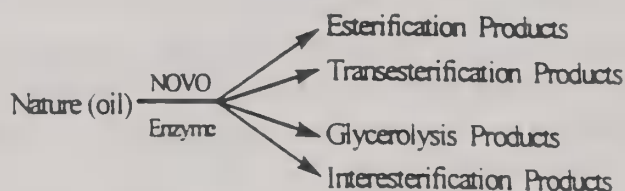


Fig. 3. Transformations of Natural Oils via Enzymatic Catalysis in SC-CO₂.

Further exploration by King and Jackson (1997) showed that under the same experimental conditions, the Novozym SP 435 catalyst could initiate glycerolysis of vegetable oils to yield monoglyceride-containing mixtures varying in composition depending on the reaction conditions. Key parameters in controlling the resultant monoglyceride concentration in the end product were the water content of the polyol participating in the reaction, the flow rate of the reactants, and supercritical fluid. Monoglyceride compositions approaching 90 wt. % can be achieved by this method. Surprisingly the reaction appears to take place in a multi phase system since typical polyol compositions exceed their equilibrium solubility levels in SC-CO₂. This invention is the subject of a U.S. patent application (Jackson, 1996) that has recently been granted.

Along a similar theme, Jackson, et al. (1997) demonstrated that the above lipase could also effectively randomize vegetable oils to yield potential products for incorporation into margarine formulations. The degree of randomization attained on the vegetable oils dissolved in SC-CO₂ was a function of extraction/reaction pressure, the flow rate of the SC-CO₂, and the quantity of enzyme utilized. Dropping point and solid fat index (SFI) data of the products randomized in SC-CO₂ were compared to oils randomized by conventional methods, and the agreement between these two differently synthesized products was encouraging. As shown in Figure 4, randomized palm olein (PO) and a genetically-engineered soybean oil randomized in SC-CO₂ (HS-1) had similar SFI as a function of temperature to hydrogenated blends of vegetable oils. This illustrates how a customized oleochemical-derived product can be synthesized using a reaction conducted in supercritical fluid media.

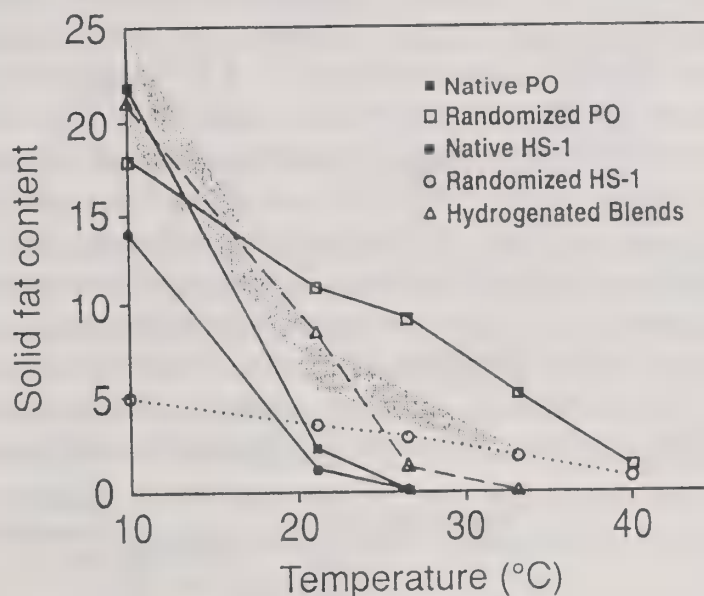


Fig. 4. Solid Fat Content of Palm Olein (PO) and HS-1 Soybean Oil (Jacob Hartz Seed Co., Stuttgart, AR) Before and After Randomization.

Another synthetic option we have explored is using heated, compressed water (subcritical water) for hydrolyzing fats and oils to produce mixtures of fatty acids for industrial use (Holliday, et al., 1997). This is very similar to the traditional "fat-splitting" processes currently used in industry except the ratio of water/fat in our process is about 2.5 versus a ratio of 0.6-0.8 commonly used in industrial fat splitters. One can achieve over 97% conversion of the starting vegetable oils to their component fatty acids using a dynamic flow system in about 10 minutes employing temperatures of 260-280°C. Pressures are typically low, 12 - 15 MPa, under these conditions, however the resultant fatty acid mixtures form an emulsion in the aqueous extract requiring further separation. As will be discussed later, we believe that this is an excellent candidate for employing a tandem process consisting of subcritical water reaction conditions followed by liquid- or SC-CO₂ extraction of the fatty

acids from the aqueous emulsion.

Supercritical Fluid Fractionation of Oleochemical-Derived Extracts and Reaction Products

As noted in the introduction, fractionation techniques employing supercritical fluids can be employed to further enrich a supercritical-derived extract or reaction product, or be applied to neat oils to enrich a particular desired component. With respect to the latter operation, we conducted research at NCAUR designed to win high value ingredients from vegetable oils, such as tocopherols, phospholipids, and sterols. In a previously reported study (King, et al., 1996) have shown that by careful control of SFE parameters, it is possible to produce an enrichment of tocopherol components in a soybean oil extract. This is achieved because of the differential extraction rates of tocopherols relative to the triglycerides in the oil at 25 MPa and 80°C which produces enrichment factors relative to their concentration in soybean ranging from 1.8 - 4.3. To provide further enrichment, these researchers further employed preparative scale supercritical fluid chromatography (SFC) on the extracts from the SFE stage using a commodity, low price silica gel. After screening sets of extraction conditions, further enrichment was affected by conducting SFC at 25 MPa and 40°C. After this operation was performed, enrichment factors rose from 2.5 to 31 depending on the individual tocopherol. This type of tandem enrichment process shows considerable promise for isolating high value phytochemicals from vegetable oil matrices without introducing solvent residuals into the oil matrix. Similar studies of this type are also ongoing at NCAUR utilizing SC-CO₂/ethanol cosolvent mixtures for fractionating vegetable oil-derived lecithins into their phospholipid components utilizing sequential SFE and SFC.

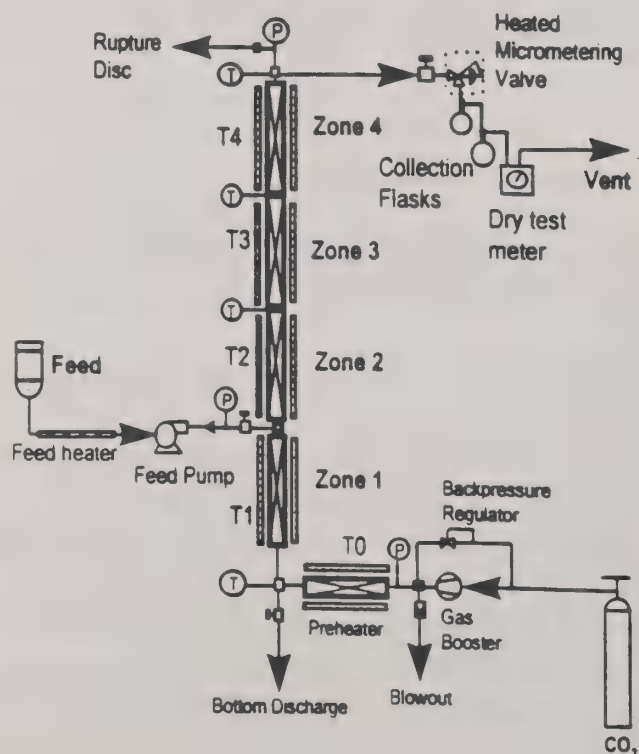


Fig. 5. Schematic of the packed column fractionation system.

Another supercritical fluid based fractionation process that is currently being used at NCAUR is the use of a thermal gradient fractionating tower for enriching monoglyceride-containing mixtures. In these studies we have used the semibatch fractionation approach of Nilsson, et al. (1988) who fractionated fish oil esters for nutraceutical use with this procedure. In our experiments (King, et al., 1997b), a packed 56 cm long column (1.43 cm in diameter) was used at pressures between 20 - 35 MPa, incorporating a thermal gradient ranging from 65 - 95°C (see Figure 5) to enrich the monoglycerides in reaction mixtures containing all glyceride species. Hence, glycerolysis products from either the CO₂ or enzyme-initiated glycerolysis can be further enriched and purified by this method. Fractionating efficiency is an acute function of glyceride throughput and pressure, the optimal fractionation occurring at lower pressures (17.5 MPa). Enrichment to monoglyceride levels between 90 - 95 wt. % is possible by this method, equal those attained by molecular distillation. It is possible therefore to envision a process whereby the raffinate in this fractionation scheme could be returned to a reaction process and be further converted to monoglycerides via a SFR.

Integrated Supercritical Fluid-Based Processing

The above examples taken from our research at NCAUR illustrate the expanding role and possibilities that supercritical fluid-based processing potentially offer the oilseeds processing and converting industry. Although the concept of an integrated SFE-SFF-SFR processing scenario may seem somewhat futuristic, it does offer the industry a range of possibilities for environmental compliance and to produce superior and value-added products from their feedstocks. As illustrated above in integrating a SFR sequence (glycerolysis using SC-CO₂) with a SFF step (fractionation of monoglycerides), there are several other tandem processes that would allow the use of sub- and supercritical fluid media to process oleochemicals. Several of these options are enumerated below:

Ester Synthesis via SFR/Ester Fractionation by SFF
Fatty Acid Production via SFR/Fatty Acid Isolation by SFE
Phospholipid Isolation by SFE/Phospholipid Enrichment by SFC
Ester Production via SFR/Ester Hydrogenation by SFR

The last option is particularly interesting since it advocates conducting hydrogenations in supercritical fluid media. Several reports (Tacke, et al., 1996; Harrod and Moller, 1996) have appeared that describe the hydrogenation of fats and oils under supercritical conditions, particularly in continuous flow reactor systems exhibiting high throughput and fast conversion efficiencies. The basis of this reported performance again lies in the superior mass transport properties exhibited by reactants in supercritical fluids, particularly the ability to enhance contact between the hydrogen, catalyst moiety, and lipid specie under these conditions. The recorded low trans content of oils hydrogenated under these conditions is encouraging, but the oilseed processing industry would have to be willing to consider retooling for this new processing option.

Impact of Analytical Supercritical Fluid Based Methodology on the Oilseed Processing Industry

Unfortunately time and space limitations limit a thorough discussion of the impact of analytical supercritical fluid methodology and its implications for the oilseed processing industry, but the author would be remiss not to mention developments in this area, including analytical research at NCAUR which have formed the basis of current developments. A nice summary of the research to date has been provided by King (1998). Utilizing SC-CO₂, it is now possible to determine the oil content of seeds using analytical SFE (Taylor, et al., 1993). Recently a collaborated method has been approved by the AOCS and it is described by King and O'Farrell (1997). Based on our previously described synthetic research, we have developed a unique and highly specific method for determining lipid levels in a variety of food matrices, including oilseeds (Snyder, et al., 1997). This automated analytical method evolved from our process studies to enzymatically convert seed oils to biodiesel in SC-CO₂ media, as depicted in Figure 6.

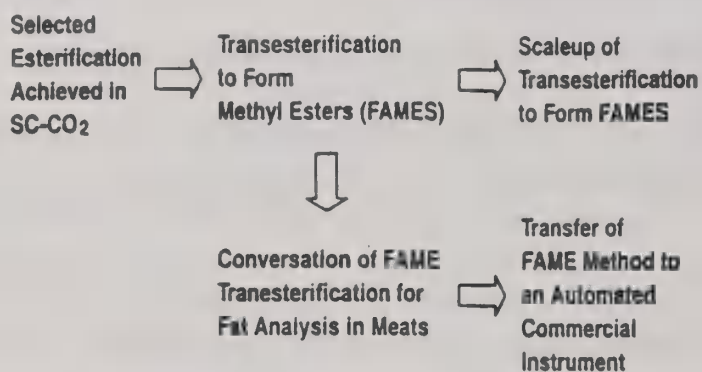


Fig. 6. Development and Utilization of Lipase Reaction in SC-CO₂.

Recently this approach has been employed to determine the total fatty acid content of commercial soapstocks (King, et al., 1998). This method is summarized in Figure 7, where the SFE/SFR-based method is compared to AOCS Official Method G3-53, a titrimetric and solvent extraction-based method. Clearly the supercritical fluid based method is quicker to perform and substantially reduces the quantity of solvent required in the laboratory environment. We further believe that the method is more accurate than the conventional method for determining the fatty acid content of soapstock (Method G3-53), since the lipid content of the soapstock is based on a rigorous gas chromatographic-fatty acid methyl ester conducted after the SFE/SFR has been performed on the soapstock sample. It is also interesting to note in Figure 7, that a capillary SFC-based method provides an approximate analysis of the fatty acid content of the soapstock in 45 minutes while using minimal solvent. This is again another example of the versatility of supercritical fluids and the promise they hold for the oilseed processing industry.

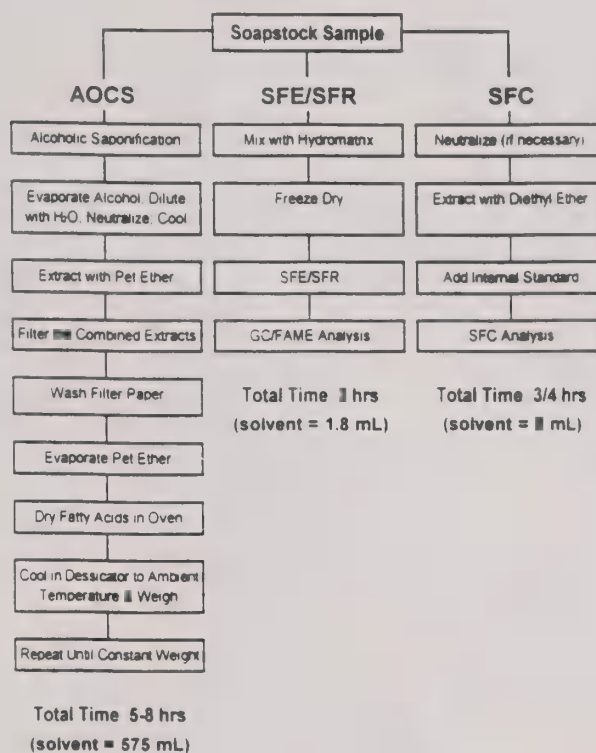


Fig. 7. Comparison of AOCS Official Method G3-53 with the SFE/SFR and SFC Methods.

References

- Anastas, P.T. and Williamson, T.C., eds., *Green Chemistry*, 1996, American Chemical Society, Washington, DC.
- Eggers, R., *J. Am. Oil Chem. Soc.*, **1985**, 62, 1222-1230.
- Friedrich, J.P. and List, G.R., *J. Am. Oil Chem. Soc.*, **1982**, 58, 192-193.
- Harrod, M. and Moller, P. In *High Pressure Chemical Engineering*, vonRohr, V.R. and Trepp, C., eds., 1996, Elsevier Science, Amsterdam, pp. 43-48.
- Holliday, R.L., King, J.W. and List, G.R., *Ind. Eng. Chem. Res.*, **1997**, 36, 932-935.
- Jackson, M.A. and King, J.W., *J. Am. Oil Chem. Soc.*, **1996**, 73, 353-356.
- Jackson, M.A. and King, J.W., *J. Am. Oil Chem. Soc.*, **1997**, 74, 103-106.
- Jackson, M.A., King, J.W., List, G.R. and Neff, *J. Am. Oil Chem. Soc.*, **1997**, 74, 635-639.
- Jackson, M.A. U.S. Patent Application 08/679,368, 1996.
- King, J.W., Favati, F. and Taylor, S.L., *Sep. Sci. Tech.*, **1996**, 31, 1843-1857.

King, J.W. and List, G.R., eds., *Supercritical Fluid Technology in Oil and Lipid Chemistry*, **1996**, AOCS Press, Champaign, IL.

King, J.W., Holliday, R.L., Sahle-Demessie, E., Eller, F.J. and Taylor, S.L., Proc. of the 4th International Symposium on Supercritical Fluids, **1997a**, May 7-11, Sendai, Japan, pp. 833-838.

King, J.W. and O'Farrell, W.V., *INFORM*, **1997**, 8, 1047-1051.

King, J.W., Sahle-Demessie, E., Temelli, F. and Teel, J., *J. Supercrit. Fluids*, **1997b**, 10, 127-137.

King, J.W., *J. Assoc. Off. Anal. Chem. Int.*, **1998**, 81, 1-9.

King, J.W. Taylor, S.L., Snyder, J.M. and Holliday, R.L., *J. Am. Oil Chem. Soc.*, **1998**, Submitted for publication.

List, G.R., King, J.W., Johnson, J.H., Warner, K. and Mounts, T.L., *J. Am. Oil Chem. Soc.*, **1993**, 70, 473-476.

Nilsson, W.B., Gauglitz, Jr., E.J., Hudson, J.K., Stout, V.F. and Spinelli, J., *J. Am. Oil Chem. Soc.*, **1988**, 65, 109-117.

Reverchon, E. and Osseo, L.S., *J. Am. Oil Chem. Soc.*, **1994**, 71, 1007-1012.

Stahl, E., Quirin, K.-W. and Gerard, D., *Dense Gases for Extraction and Refining*, **1988**, Springer-Verlag, Heidelberg.

Snyder, J.M., King, J.W., and Jackson, M.A., *J. Am. Oil Chem. Soc.*, **1997**, 74, 585-588.

Tacke, T., Wieland, S., and Panster, P., Proc. of the 4th International Symposium on Supercritical Fluids, May 11-14, 1997, Sendai, Japan, pp. 511-514.

Temelli, F., King, J.W. and List, G.R., *J. Am. Oil Chem. Soc.*, **1996**, 73, 699-706.

*Alternative Methods for Formulation
of Food Oil Products*

Gary R. List
NCAUR/USDA/ARS
Peoria, IL



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Alternative Methods For Formulation Of Food Oil Products

G.R. List, Food Quality and Safety Research, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, 1815 N. University Street, Peoria, IL, 61604

Current research is focused on developing new technologies offering alternatives to partial hydrogenation thus eliminating trans fatty acid isomers in food oils intended for margarines, spreads, and shortenings. Random interesterification of liquid vegetable oils with harder components was shown to be a versatile tool for preparation of margarine and spread oils having suitable solid fat index profiles and melting points. Similarly, shortening basestocks can be prepared from interesterification of palm oil with vegetable oil flakes. Blending of these products with liquid vegetable oils allows formulation of shortening oils with a wide range of solids content. Traditionally, random interesterification of vegetable oils has been carried out in the presence of chemical catalysts most notably with sodium methoxide. Studies were conducted in which vegetable oils were randomized in flowing supercritical carbon dioxide using an immobilized enzyme bed. By varying pressure, CO₂ flow, and enzyme concentration, the reaction can be controlled to yield products with the desired physical properties. Soybean oils high in stearic acid have been developed by genetic breeding and their utility in margarine oil has been investigated. In their natural states, oils containing 17-33% stearate are unsuitable over the entire (10-33.3°C) temperature range required of margarine oils. However, after blending with harder components, these oils showed promise as either soft tub or stick margarine oils. Interesterification of high stearic oils either with sodium methoxide or with immobilized lipase in flowing CO₂, alters their solid fat index profiles and melting points to such an extent that blending with tropical components becomes unnecessary.

Names are necessary to report on available data; the USDA neither guarantees nor warrants the standard of the product and the use of the name by USDA, implies no approval of the product to the exclusion of others that may be suitable.

Historical Perspective

Today's consumers demand spreads that are low in fat and cholesterol and the industry has responded with a wide array of stick, soft tub, squeezable, and spray products. Indeed, since 1980, the fat content of margarine has decreased from 80% to an average of 56% whereas other products are available containing from 0 to 68% fat. Over the past 25 years, a number of controversial reports have appeared linking fats, oils, and trans acids to elevated serum cholesterol levels (Emken, 1995). Recently, several spread products have been introduced that were formulated with plant sterol esters or corn fiber oil and were claimed to lower cholesterol levels in humans. Trans acids formed during partial hydrogenation of edible oils have prompted alternative methods to formulate margarine spreads and shortenings. Zero trans margarines and spreads have been available in Europe and Canada for a number of years and recently at least two U.S. processors have reformulated product lines to be trans free (Haumann, 1998). This paper will review some of the work conducted at NCAUR on alternative methods to formulate food oil products such as margarines (List et al., 1995_a, 1995_b, 1996, 1997; Jackson et al., 1997).

Margarine and Shortening Oils by Interesterification of Liquid and Trisaturated Triglycerides

The concept of randomly interesterifying liquid vegetable oils and stearine was recently reported to be a versatile tool for preparation of margarine and shortening oils (List et al., 1995). Common vegetable oils including soybean, corn, cottonseed, peanut and canola when randomized with 20% soybean or cottonseed stearines possess solid fat index (SFI) profiles and melting points comparable to hydrogenated oils blended for soft tub margarines. For example, hydrogenated tub margarine oils show SFI values at 10, 21.1, 33.0°C ranging from 8-10, 4-5, 1-2 respectively with dropping points of 32.2-35.6°C. Interesterified 80:20 liquid oil-stearines show comparable values. Shortening oils require higher, flatter SFI curves and melting points than margarine oils. In order to achieve these properties, it becomes necessary to incorporate more stearine into the interesterified blend to form a base stock. The desired SFI profiles are achieved by blending with additional liquid oil. The effect of blending liquid soybean oil with an interesterified shortening base stock prepared from a 50:50 mixture of liquid soybean and soybean stearine are shown in Figure 1.

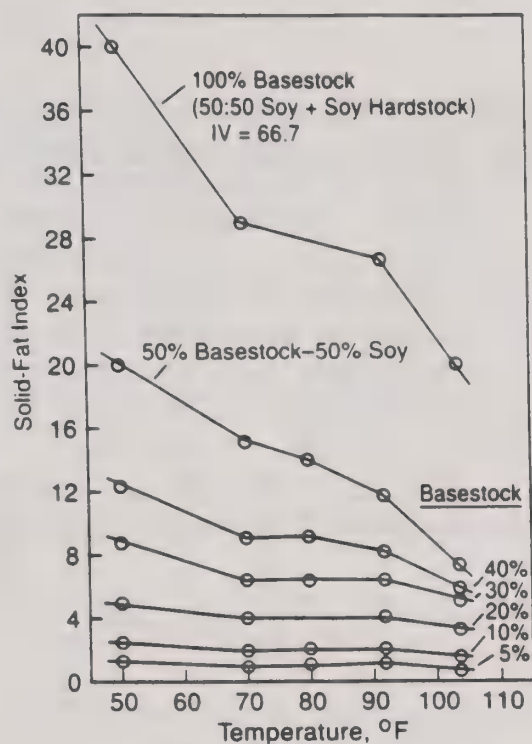


Figure 1. Effect of liquid soybean oil on the SFI profile of an interesterified soybean-stearine blend (50:50).

Typically, all purpose shortening oil prepared by blending hydrogenated stocks show SFI values at 10, 21.1, 26.7, 33.3 and 40°C of 18-23, 14-19, 13-14, and 7-11 respectively. Results shown in Figure 1 show that a blend of 50% base stock and 50% liquid soybean oil have an SFI curve closely matching these values. Commercial oils have drop melting points of 45-47.8°C whereas the 50:50 blend value of 42.2°C is slightly lower. Thus the concept of formulating all purpose shortening oils by blending interesterified basestocks with liquid oils appears valid. Fluid shortening oils represent products with low, flat SFI curves with values ranging from 8 at 10°C to 6 at 40°C. Oils prepared by blending 35% of the 50:50 basestock with 65% liquid corn, peanut, cottonseed, or canola possess SFI profiles closely matching that of fluid type shortenings.

Preparation and Properties of Zero Trans Soybean Oil Margarine

Preliminary pilot plant studies on the formulation of zero trans margarines made from interesterified soybean oil stearine blends have been reported (List et al., 1995) and compared to products formulated with hydrogenated soybean oil (iodine value=107). Soybean oil and soybean stearine were blended in a 4:1 ratio and rearranged with sodium methoxide (0.3%) at 100°C; the catalyst was neutralized and the oil was washed with warm water, then dried, bleached, filtered and deodorized. Margarines were formulated with 80% fat with 0.2% emulsifier and 0.1% lecithin. The fat phase was brought to 57°C in an emulsion tank and the aqueous milk phase was slowly added until the temperature reached 49°C. The margarines were prepared by pumping the emulsion through 2 "A" votator units operating at 400 rpm. The product coming from the "A" units was passed through a blender or "B" unit operating at 200 rpm. Product coming from the "B" unit was then passed through another "A" unit after which it was poured into plastic tubs before being placed in

storage at 4.4°C. Exit temperatures for the 3 "A" units were 25.6-26.6 °C, 13.3-13.4°C, 6.7-7.8°C. Properties of the experimental margarines are shown in Table 1.

TABLE 1

Properties of Experimental Margarines

Sample	Oil component	Penetration ^a × 1/10 mm	Yield value g/cm ²	Water loss (mL)		Oil loss (mL)		Water (%)	Salt (%)	LFRA ^b
				21.1°C	30°C	21.1°C	30°C			
Pilot-tub	Hydrogenated soybean oil	187	407	0	0	0	2.7	16.7	1.7	80
Pilot-tub	Interesterified soybean oil ^c	70	1961	0	0	0	3.1	16.6	1.8	160
Pilot-Tub	80% Interesterified 20% Soy oil	137	670	0	0	0	1.0	16.3	2.1	120

^aAOCS Method Cc 16-60

^bTexture reading, LFRA = Leatherhead Food Research Association, 40° cone, 5-g load, 4-mm drop, 1/2 mm/s.

^c80:20 Soy and soy tristearin.

A survey of commercial tub margarines showed penetration values ranging from 155-279 that equate to yield values of 217-539 g/cm². Although the spreadability range of margarines equates to yield values in the 200-800 gm/cm² (Haighton 1959), most premium soft table margarines formulated from hydrogenated soybean oil show yield values in the 200-550 g/cm² range. Soft spreadable stick and stick products show yield values in the 960-1360 and 1680-2800 gm/cm² range respectively. Margarine prepared from the interesterified 80:20 blend showed a yield value of 1961 g/cm² which was spreadable, but was too hard for a soft tub product and more in line with stick products. The 80:20 margarine had a slight tendency to pull away from the tub after filling and after final 4.4°C storage. However, blending the 80:20 feedstock with an additional 20% liquid soybean oil alleviated this problem and resulted in a softer product having a yield value of 670 g/cm². The experimental margarines were subjected to an oil off test (Sieden 1965) that includes storage for 4 days at 21.1°C and 1 day at 30°C. No oil or water loss was observed after 4 days storage at 21.1°C whereas after 1 day at 30°C no water loss was evident and only small oil loss occurred. These results indicate that the interesterified margarines performed as well or better than the hydrogenated control.

Spreadability was rated by a trained panel on a 10 point scale with 0=soft and 10= hard. The hydrogenated control, 80:20 interesterified and the 80:20 + 20% liquid oil scored 1.3, 2.1, and 0.6 respectively for spreadability. Graininess was scored on a 10 point scale with 0=smooth and 10=grainy. Essentially no graininess was observed in any of the experimental margarines. Mouth melt time was measured by placing a 3 gm sample into the mouth directly out of the refrigerator and measuring the time required for complete melting. The hydrogenated control showed a time of 18 seconds whereas the interesterified and interesterified + 20% liquid oil showed times of 23 and 15 seconds respectively. These values

are in excellent agreement with published literature values (Vaisy-Genser and Vane, 1995). Results presented here suggest that interesterified fats crystallize more slowly than hydrogenated oils processed under the same conditions. Margarine emulsions that crystalize slowly in the absence of agitation (after filling into tubs) favor the formation of a strong network between water, fat crystals and liquid oil leading to harder products (Haighton, 1976). Nonetheless results with suitable properties and emulsion stability can be produced from interesterified soybean oil.

Potential Margarine Oils from Genetically Modified Soybean Oil

Over the past few years, a number of genetically modified soybeans have been developed having elevated palmitic and stearic acid contents. Compared to the typical 15% saturated acids found in normal soybean cultivars, high saturate oils may contain up to 40% palmitic and stearic acids (Hammond and Fehr, 1983; Wilson and Burton, 1992; Graef et al., 1985). The physical properties of some genetically modified soybean oils high in stearic acid are shown in Table 2.

TABLE 2

Effect of Interesterification on Physical Properties and Component Glycerides of Genetically Modified Soybeans That Are High in Stearic Acid												
Variety	State	Stearic (%)	Solid fat index @ temp. (°C) ^a					Drop point (°C) ^c	Component glycerides (%) ^b			
			10.0	21.1	26.7	33.3	40.0		UUU	UUU and USU	USS and SUS	SSS
A6	Natural	23.9	11.2	0.0	0.0	0.0	0.0	19.7	22.1	47.8	26.3	2.3
A6	Natural	27.1	22.8	12.1	0.0	0.0	0.0	18.7	15.9	39.5	40.8	2.5
A6	Natural	33.0	18.7	7.9	0.0	0.0	0.0	19.9	18.1	41.0	37.5	4.3
A6	Interest	23.9	6.8	3.3	2.8	1.5	0.6	23.2	24.9	46.1	23.8	2.2
A6	Interest	27.1	13.7	4.5	3.8	2.6	1.5	38.2	19.9	42.7	30.0	4.3
A6	Interest	33.0	13.5	5.5	4.7	3.5	1.7	36.4	18.2	40.8	35.2	3.2
HS-1	Natural	20.6	12.6	1.5	0.0	0.0	0.0	17.6	25.8	42.6	23.9	2.5
HS-1	Interest	20.6	7.8	4.4	3.4	2.6	1.2	36.8	26.6	45.1	21.1	2.9
A90	Natural	17.2	6.0	0.0	0.0	0.0	0.0	14.2	36.0	45.1	17.2	1.5
A90	Interest	17.2	3.8	2.7	1.7	1.1	0.4	30.4	37.8	44.6	14.5	2.1

^aBy AOCS method (14); interest., interesterification; U, unsaturated; S, saturated.

^bBy AOCS method (14).

^cBy HPLC (13), % of total triglycerides; A6 (Iowa State University (Ames, IA); HS-1, Jacob Hartz Seed Co. (Stuttgart, AR); A-90, Pioneer Hi-Bred International Inc. (Waterloo, IA).

Included are oils from Pioneer Hybrids (A-90), Iowa State University (A-6), and Hartz Seed Co. (HS-1). In their natural state, none of these oils qualify as either a soft tub, stick, or liquid margarine oil. They lack enough solids at 21.1 or 33.3°C and their melting points are too low. It became obvious that if genetically modified high stearic acid soybean oils were to be used in margarine formulations, blending with harder components such as palm oil, interesterified palm oil, or vegetable oil stearines would be needed. Indeed, studies made in which Pioneer A-90 oil was blended with either 40% palm oil, or interesterified palm oil, or 10% of a palm-soy interesterified base stock, SFI profiles and drop points were comparable to hydrogenated oils formulated for soft tub

margarine. The A-6 and HS-1 oils when blended with 2-3% vegetable oil stearine qualified as a stick margarine oil. Also shown in Table 2 are the effects of interesterification on the physical properties of the genetically modified soybean oils. The results indicate that after randomization, marked differences in SFI and melting point occur such that blending with tropical oils becomes necessary. It is believed that since palmitic and stearic acids are found almost exclusively in the outer 1-3 positions in the natural triglycerides, the SUS types predominate whereas after randomization, more of the higher melting SSU types are formed.

Enzymatic Interesterification of Triglycerides in Flowing CO₂

Another area of research we have pursued involves enzymatic interesterification of triglycerides in flowing CO₂ (Jackson et. al. 1997). Triglycerides are absorbed on celite in a tubular reactor and supercritical carbon dioxide at 4000 psi is passed through the substrate where the triglycerides are solubilized in the gas phase after which it enters the reactor containing a commercially available enzyme, Novzym 435 maintained at 65°C. Enzymatic interesterification shows some promise as a route to margarine oils. By varying operational parameters such as CO₂ flow, pressure, temperature, and catalyst concentration, products with tailor-made physical properties can be prepared. Under optimum conditions, products obtained are comparable to those obtained by chemical interesterification with sodium methoxide catalyst. Although interesterification and genetically modified oilseeds high in stearic acid show great promise as alternative methods to prepare margarines, shortenings, and spreads, much basic research remains to be done. In particular, new knowledge concerning the chilling and crystallization of emulsion containing interesterified and genetically modified oils requires study. Problems of post hardening, water bleed, graininess, texture defects, spreadability, carry over of salt from the aqueous phase, and poor mouth melt properties require more research and are currently under investigation.

References

- Emken, E. A., *J. Clin. Nutr.*, **1995**, 62, 6655-7085.
- Haighton, A.J., *J. Am. Oil Chem. Soc.*, **1976**, 53, 397.
- Haighton, A.J., *J. Am. Oil Chem. Soc.*, **1959**, 36, 345.
- Hammond, E.G. and W.R. Fehr, *Crop Sci.*, **1983**, 23, 192.
- Haumann, B.F., *INFORM*, **1998**, 9, 6-11.
- Graef, G.L., Miller, L.A., Fehr, W.R., and Hammond, E.G., *J. Am. Oil Chem. Soc.*, **1985**, 62 773-775.

Jackson, M.A., King, J.W., List, G.R., and Neff, W.E., *J. Am. Oil Chem. Soc.*, **1997**, 24, 635-639.

List G.R., Mounts, T.L., Orthoefer, F., and W.E. Neff, *J. Am. Oil Chem. Soc.*, **1995**, 79-382.

List, G.R., Pelloso, T., Orthoefer, F., Chrysam, M., and Mounts, T. L., *J. Am. Oil Chem. Soc.*, **1995**, 72, 383-384.

List, G.R., Mounts, T.L., Orthoefer, F., and Neff, W.E., *J. Am. Oil Chem. Soc.*, **1996**, 73, 729-732.

List, G.R., Mounts, T.L., Orthoefer, F., and Neff, W.E., *Ibid.*, **1996**, 74, 327-329.

Wilson, R.F., and Burton, J.W., *Proceedings World Conference on Oilseed Technology and Utilization*, **1992**, AOCS Press, Champaign, IL, 422-429.

Sieden, P., *Canadian Patents*, **1965**, 718, 372.

Vaisey-Genser, M., and Vane, B.R., In *Methods to Assess Quality and Stability of Oils and Fat Containing Foods*, Ed. K. Warner and N.A. Michael Eskin, **1995**, AOCS Press, Champaign, IL, 76-106.

*Minimizing Environmental Costs
and Maximizing Compliance*

Michael J. Boyer
Applied Engineering and Science
Atlanta, GA



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

ABSTRACT

MINIMIZING ENVIRONMENTAL COSTS AND MAXIMIZING COMPLIANCE

Michael J. Boyer, P.E.

President

Applied Engineering and Science, Inc.

Atlanta, Georgia

Presented at the 47th Oilseed Conference

March 1998

New Orleans, Louisiana

Effective environmental management is based on development of a well-designed and implemental program at both the plant and corporate level. Minimizing cost and maximizing compliance will be a natural outcome of program implementation.

The ability to measure success is critical to program management. This must include elements of cost and effectiveness. Development and implementation of a corporate environmental policy document is essential as the managing tool for program direction. A plant environmental audit program is also essential.

Several specific items for both plant and corporate levels are discussed. The key component of any item is that it be actionable, and that goals be reasonably attainable and measurable.



MINIMIZING ENVIRONMENTAL COSTS AND MAXIMIZING COMPLIANCE

Michael J. Boyer, P.E.

President

Applied Engineering and Science, Inc.

Atlanta, Georgia

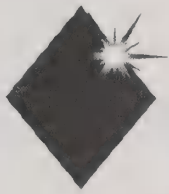


Presented at the 47th Oilseed Conference

March 1998

New Orleans, Louisiana

Notes:



Money

(You Have to Spend Money to Make, or Save, Money)

- ◆ Short Term Spending
- ◆ Indirect Costs
- ◆ Savings in Product Loss and Energy Consumption
- ◆ Lost Product Sales Due to Bad Publicity

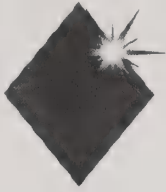
Notes:



How Do You Measure Success

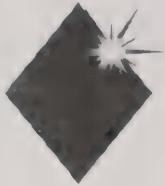
- ◆ Money
- ◆ Effectiveness

Notes:



The best way to spend the most money and get the least results in the environmental control area is to ignore the area (or have a totally inadequate program).

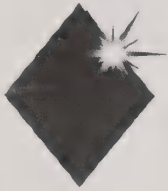
Notes:



Environmental Cost Measurement

- ◆ Corporate Overhead Costs
- ◆ Plant Costs
- ◆ Incremental Product Costs
- ◆ Secondary Costs

Notes:



Management of Environmental Information

- ◆ Develop Actionable Goals Based on Money and Effectiveness
- ◆ Develop Information Feedback Systems

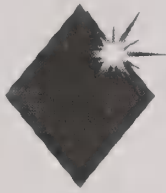
Notes:



Effectiveness

- ◆ Regulation Compliance
- ◆ Meet Corporate Environmental Policy
- ◆ Perceived “Green” Image From Clients and Constituents
- ◆ Expedite New Facilities Construction
- ◆ Other

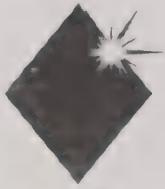
Notes:



Corporate Level

- ◆ Develop Financial Measurement
- ◆ Develop and Implement Environmental Policy and Auditing Program
- ◆ Move Decision Making Downward (With Resources)

Notes:



Plant Level

- ◆ Sample Analysis and Costs
- ◆ Permits - What Constitutes Compliance
- ◆ Frequent Short Seminars
- ◆ Actionable Goals at Plant Level
- ◆ Management Challenge - Work Smarter not Harder
- ◆ Read, Understand and Publicize Corporate Environmental Policy

Notes:

*Impact of OSHA 1910.119 on Automation
in Solvent Extraction Plants*

Mark B. Strube
Process Systems, Inc.
Memphis, TN



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

**Impact of OSHA 1910.119
on Automation in
Solvent Extraction Plants**

**Mark B. Strube PE
Project Engineer**



**PSI, Process Systems, Inc.
1790 Kirby Parkway
Suite 300
Memphis, TN 38138
(901) 756-8250**

Table of Contents

1. WHAT IS OSHA STANDARD 1910.119.....	1
1.1 INTRODUCTION TO THE PSM STANDARD	1
1.2 WHY YOU NEED TO KNOW	1
2. COMPLIANCE DOCUMENTATION.....	2
2.1 PROCESS SAFETY INFORMATION	2
2.2 PROCESS HAZARDS ANALYSIS	3
2.3 OPERATING PROCEDURES.....	4
2.4 EMERGENCY PLANNING AND RESPONSE.....	5
3. MANAGEMENT OF CHANGE PROCEDURES	5
3.1 CONTROL SYSTEM SECURITY	6
3.2 REVIEW AND AUTHORIZATION REQUIREMENTS	6
4. TRAINING REQUIREMENTS.....	7
4.1 INITIAL AND REFRESHER TRAINING	7
4.2 SIMULATION BASED TRAINING	7
5. CHECK OUT PROCEDURES.....	8
5.1 PRE-STARTUP SAFETY REVIEW	8
5.2 MECHANICAL INTEGRITY REQUIREMENTS.....	9
5.3 PROCESS AUTOMATION COMMISSIONING	9
6. SUMMARY	10
6.1 FOR MORE INFORMATION	10
6.2 ABOUT THE AUTHOR	10

1. What is OSHA Standard 1910.119

The Occupational Safety and Health Administration (OSHA) as a part of the U.S. Department of Labor is in charge of making sure that employees are able to perform their job functions in the safest way possible. In February 1992, OSHA published standard 1910.119 titled "Process Safety Management of Highly Hazardous Chemicals". This standard went into effect in May of 1992, and specifically deals with the management of process safety systems which employers have to implement to protect their employees from toxic, reactive, flammable, or explosive chemicals in the workplace. Process safety systems required by the rule include detailed information about the process and the hazards of its operation, operating procedures, training requirements, startup and checkout requirements, management of change procedures, and plans for handling hazards when they occur in the workplace.

1.1 Introduction to the PSM Standard

The Process Safety Management (PSM) standard applies to processes with more than 10,000 pounds of a flammable liquid or gas on a site in a single process. Specific chemicals listed in Appendix A to the OSHA standard have significantly lower threshold quantities (TQ). The regulation defines a process as any activity involving a highly hazardous chemical (HHC) including any use, storage, manufacturing, handling or the on-site movement of such chemicals or a combination of these activities. OSHA extends the boundaries of a process by including all piping and vessels which are interconnected or are located such that a highly hazardous chemical could be involved in a potential release.

Exceptions are made for sites where the threshold quantity is exceeded for hydrocarbon fuels or where the flammable liquid is stored in an atmospheric tank and kept below its normal boiling point without benefit of refrigeration or chilling. Specifically exempted from the rule are retail facilities, oil and gas drilling or servicing facilities, and normally unoccupied remote facilities. The exemptions for other types of facilities, including solvent extraction facilities, are canceled when the process in question is located such that it may be affected by a catastrophic release in another area. In general, storage tanks connected to the actual process equipment where the highly hazardous chemicals are used are considered part of the process, since they can be affected by catastrophic releases within the primary process vessels. In these cases, storage tanks become part of the process, and are no longer used purely for storage, which makes the boiling point of the HHC irrelevant. Further, this would also cause the threshold quantity of HHCs to be exceeded, making it near impossible to justify an exemption from PSM regulations.

1.2 Why You Need to Know

The most common solvent used in today's solvent extraction facilities is various forms of hexane. Hexane is extremely flammable with an NFPA rating of 3, and is considered a highly hazardous chemical by OSHA regulations, but is not specifically listed in the Appendix A to the PSM regulation. Therefore, any solvent extraction facility with a process containing more than 10000 pounds (about 1800 gallons) of hexane is required to abide by the regulations set forth by OSHA 1910.119. The threshold quantity of 10,000 pounds need not be in the primary process equipment, but any equipment directly piped to the primary process equipment including day tanks and storage tanks, as these may be affected by catastrophic releases.

Being exempted from the requirements of the rule is not impossible but will be very difficult. In fact, it will probably be less trouble to comply with the PSM regulations than to try to avoid them. A solvent extraction facility would have an especially difficult time being exempted. It is unlikely that a modern plant could operate profitably without exceeding the threshold quantity of hexane or some other solvent. A plant would have to have less than 10000 pounds of solvent on the site, including bulk storage, and

would have to maintain records to prove that the TQ is never exceeded, even temporarily. Another possible exception would come under operation in a normally unoccupied remote facility. This can't be a separate building in the back corner of your existing property. It must be a separate site, not close to anything, and must be able to operate without any personnel, with only periodic maintenance. OSHA would cancel this exception for any facility which could pose a threat to any personnel or adjacent facilities if there were a catastrophic release of HHCs. Further, the expense of constructing, operating, and maintaining a "lights out" facility that would pass OSHA muster would be significantly more expensive than adhering to the regulations.

So now that you've decided you can't avoid the regulation, what do you have to do to comply, and specifically, how will the regulation affect the automation of your facility. The PSM regulation does not restrict how much or how little automation exists in your facility, but does require that you know and document exactly what that automation does. You must train all personnel on how to operate the facility safely, what to do if something goes wrong, and be able to prove that they still know what you taught them. You must take extra care in checking out new construction, and document methods and results of your checkout procedures. You must have formal procedures for making changes to process control software, and you must be able to enforce them with appropriate security.

This sounds like a lot of work to satisfy a government agency. Between October 1995 and September 1996, OSHA issued 591 citations for violation of the PSM standard during 112 inspections, and levied penalties of \$2,792,636. This makes the average penalty per citation \$4,725, but since an inspection usually yields over 5 citations, the average penalty per inspection is almost \$25,000, with the maximum penalties easily reaching into six digits. Further, if you're cited, the courts can force you to comply with the regulations anyway, since the OSHA regulation is a requirement of the Clean Air Act Amendments enacted by Congress in 1990. So if you're not following the OSHA guidelines, your plant is not as safe for you or your employees as it could be. After all, safety is what OSHA is all about.

2. Compliance Documentation

To pass an inspection of your PSM program, you will need to have all of your documentation in order. The inspection checks your program in three phases termed Program-Quality-Verification (PQV). First, your compliance safety and health officers (CSHOs) will ask for access to all your PSM documentation to be sure you have a program in place. Second, the CSHO will compare the quality of the program with acceptable industry practices. Finally, the verification stage determines if your implementation of PSM is effective by reviewing written programs and records of activity, interviews with employees at different levels, and observation of site conditions. The CSHOs follow a directive published by OSHA regarding compliance guidelines and enforcement procedures. The inspection procedures outlined attempt to allow the inspector to answer yes/no questions regarding your program, with each "no" representing a citation for failure to comply. It is therefore in your best interest to have all the right documentation on hand to keep the CSHO impressed.

2.1 Process Safety Information

The basis for much of the documentation required by the PSM regulations is the process safety information. This process safety information should be used as the basis for subsequent process hazards analysis, and is usually compiled by the technical personnel that designed the process in question. The process safety information covers three basic areas, information pertaining to the hazards of the HHCs themselves, information pertaining to the technology of the process in question, and information pertaining to the equipment used in the process. Information about the equipment is the key area which impacts automation systems. Equipment information required to satisfy this section of the regulation includes materials of construction, piping & instrument diagrams (P&IDs), electrical classification, relief

system design and design basis, ventilation system design, and safety systems. Safety systems include many control system functions like interlocks and detection and suppression systems. During a PQV inspection, OSHA will request process narrative descriptions to satisfy this part of the review.

The process narratives should explain the safety systems and other interlocks of the automation systems in detail adequate to conduct a process hazard analysis. All effects on each controlled piece of equipment should be detailed. For example, the action of a specific pump might be that it is manually started by an operator, and automatically shuts down when a tank reaches a high level, or it can be much more complicated for example, a valve opens automatically to fill a vessel when it drops to low level, closes automatically when a high level is reached, and can only be opened manually with engineer's or supervisor's password. Note that the override of an interlock cannot be taken casually, and the consequences of overriding the interlock must be clearly documented, especially if there may be a safety threat, as this may effect the process hazards analysis.

Another key element of the process narratives includes information about hazard detection and suppression systems. This area of information centers around the control system's alarming functions. Alarms should be generated by the most reliable equipment and enunciated in a clear and concise fashion to all facility personnel. In PLC based systems, alarms would be generated in the PLC, while using a PC based human-machine interface HMI for monitoring and annunciation purposes only. The PC based HMI should offer an alarm display to allow an operator to see prioritized alarms throughout the facility. Alarm horns and beacons should also be located such that any personnel in the facility would be warned of a hazardous situation. Beacons should be multicolored to allow for the annunciation of simple equipment alarms, potentially hazardous early warning alarms, and emergency response alarms. The rule of thumb to remember when designing alarm systems is to keep the operator informed about all alarms, and prioritize all alarms so that the most hazardous situations can be addresses first.

In solvent extraction plants, hexane detectors should be located in key areas to detect leaks. The process narrative should detail what action should be taken by the operator and by the control system when a release is detected. Hazardous release detection should have a minimum of two stages. The first stage should give operations personnel early warning that a leak has been detected. This alarm would allow parts of the process not directly related to problem area to continue operations under direction of the automation systems while a maintenance technician checked out the leak. A second stage alarm would go into effect when hexane levels rise to hazardous levels, at which point, all automated equipment would be shut down by the control system, and the facility's emergency response plan would go into effect.

2.2 Process Hazards Analysis

Once the process safety information is compiled, a process hazards analysis (PHA) is conducted. The process hazards analysis should be conducted as soon as possible after process safety information is available. Existing processes at the time the PSM regulations went into effect have until May 26, 1997 to have completed all PHAs. PHAs are living documents which must be revalidated at least every five years to assure that the process hazard analysis is consistent with the current process. PHAs may have to be reviewed more frequently if work processes or procedures are modified in any way (other than replacement in kind, replacing a malfunctioning valve with a working duplicate, for example) through management of change procedures.

The PHA should be conducted by a team knowledgeable in engineering and process operations, with at least one employee having process specific knowledge, and one team member familiar with the specific process hazard analysis methodology being used, usually an outside consultant used for PHA facilitation. Although not specifically called for by the PSM regulation, it is a good idea to have at least one control system engineer familiar with the process control systems included on the PHA team. The control system

engineer can answer specific questions about software interlocks and security schemes, and can explain to other team members the consequences of failure of various aspects of the automation systems.

The PHA should identify, evaluate, and control the hazards involved in the process. It is the responsibility of the employer to establish a system to address the team's findings and recommendations. All activities initiated by the PHA should be documented as part of the PHA and communicated to all operating, maintenance, and other employees whose work assignments involve the process and who may be affected by the PHA. Team findings can include any changes necessary to the process control software to minimize risks to employees. Typically, the control system engineer involved in conducting the PHA can suggest supplemental interlocking or other security schemes to minimize risks identified during the analysis of process hazards.

The analysis of the control system as it affects process hazards should address human factors including the clarity and simplicity of HMI control screens and the level of automation provided by the system. People are inconsistent by nature, and it is key to configure the control system to provide consistent direction to operators. In general, always automate simple functions which can reduce the tasks to be performed by the operator. Configure critical alarms to stand out from the background with a distinct color or flashing pattern, or have them take over the entire display until they are acknowledged. This will allow the operator more time to monitor critical functions, and will reduce the number of human factors which can contribute to process hazards.

2.3 Operating Procedures

The one area of compliance documentation that most directly affects automation systems is operating procedures. Operating procedures are not a part of, nor do they replace process safety information, but they must be consistent with process safety information. They must address steps for each operating phase, operating limits, safety and health considerations, and safety systems and their functions. Phases of operation to be addressed within the operating procedures include initial startup, normal operations, temporary operations, emergency shutdowns and operations, normal shutdowns, and startups following a turnaround or emergency shutdown.

Control systems designed for use in PSM regulated processes should include much of this information directly on the operator's control screen. Steps for each operating phase can be coded into the process control software and displayed on a control screen. The automation system can even help walk an operator through a startup or shutdown by laying out each specific step. The more instrumentation that is available within a regulated process, the more the control system can do in an automated fashion. The operator's control screen should still display all steps, but the system can automatically sequence through all steps except those which require manual intervention or confirmation by an operator. Some highly automated sequences may even request that an operator make certain entries at key points in the sequence to ensure that there is still a real person watching over the process at all times.

Information about equipment operating limits should also be available to the operator from the HMI displays. There are two major types of operating limits, discrete and analog. Discrete operating limits define the discrete state of related equipment for operation of a pump, for example, and are often considered interlocks. Each piece of discrete equipment can have an associated subdisplay which specifies the discrete operating limits. This subdisplay can offer an override function to allow operation of the equipment outside of standard operating conditions, but safe operating limits should never be exceeded. When operating limits are overridden, consequences of deviation should be displayed, and deliberate action should be required to place equipment outside of standard operating conditions. This action could be an extended set of keystrokes, password protection, or external keyswitch, depending on the severity of the consequences.

Analog operating limits define the range of values from specific instrumentation under which it is normal and safe to operate. This type of limit is more often associated with analog feedback loops traditionally controlled by single loop controllers. Today's automation systems provide the loop information but with additional detail. Loop face-plates can include discrete interlocking information as well as analog operating limits. Discrete interlocks for an analog loop might include operating state of a specific pump for a loop to operate in an automatic mode. Typically, standard operating conditions would define the high and low alarm setpoints, with safe operating limits defining the high-high and low-low alarm setpoints. Where these limits are dynamic, deviation from setpoint alarming can be used. In any case, alarm setpoints should always be displayed for the operators use. However, changing these setpoints must strictly follow management of change procedures, as they are a critical part of the process safety information.

Two other key compliance areas include the accessibility of operating procedures to employees who work in or maintain a process, and the review of the procedures to ensure they reflect current operating practice. Accessibility is guaranteed by providing operating procedures directly within the operators control displays. However, by doing so, it makes it more difficult to maintain accuracy when process safety information changes. You can help keep online operating procedures up to date by giving small rewards to operations and maintenance staff when they discover inconsistencies. Although this should not be the primary reconciliation method, it can ensure continued accuracy, and it can also get all of your employees thinking of ways to improve the safety and efficiency of current operating procedures.

2.4 Emergency Planning and Response

Emergency response planning is included in the PSM standards and defined by reference to other OSHA standards; 1910.38 - Employee Emergency Plans part (a), and 1910.120 - Hazardous Waste Operations and Emergency Response parts (a), (p), and (q). These standards go into great detail about emergency response planning. The primary compliance issues include the existence of a written emergency action plan, and that all employees are familiar with its requirements. Of primary concerns to control systems engineers is the emergency alarm system required by reference to OSHA standard 1910.165 - Employee Alarm Systems.

The emergency alarm system should have several key features to comply with regulations. The alarms should be distinctive for each purpose of the alarm, which may include multiple types such as simple equipment alarms, early warning alarms, and emergency action alarms. The emergency alarm must be capable of being heard over ambient noise and distinctively signal the need to evacuate the work area. The emergency alarm must be maintained in operating condition and should be checked every two months on non-supervised systems, and at least annually on supervised systems.

For an extra level of safety in the case of a hazardous release, consider adding a remote HMI node in a location which can be used to monitor actual process conditions after evacuation of the facility. Although not required by OSHA regulations, the expense will seem very small during an emergency evacuation situation. The remote node can be used to determine the exact cause of the evacuation alarm, and can give emergency response personnel critical information to contain the hazardous release. This also provides a backup means to monitor and/or control the shutdown of automated systems from a safe location during the emergency response to a hazardous release.

3. Management of Change Procedures

In order to maintain a safe work environment, PSM regulations require that employers review and track all modifications to equipment, procedures, raw materials, and processing conditions. Any of these types of changes can affect the safety of employees working in and around the process. Minimum requirements

for management of change procedures include: establishing written procedures to manage change; addressing the technical basis, impact on safety and health, modification to operating procedures, necessary time period to complete change, and authorizations required. All this information must be communicated to affected employees as the change is being implemented, and process safety information and operating procedures must be updated to reflect the new process conditions.

3.1 Control System Security

In order to ensure that all changes to process control software follow management of change procedures, the control system should have limited and secured access only. The initial selection of a process control system should include review of security features. Features to look for include password protected access and revision tracking. Password protection systems should be set up such that maintenance personnel can troubleshoot process problems by looking at online configurations without being granted change rights. Change rights should only be granted to a select few engineers. Passwords granting change rights should be changed regularly, and all with change right access should rotate selection of the password. This password should never be written down in a memo or anywhere else, and should be communicated verbally to those employees with change rights. As a backup to password protection, some control systems have embedded switches to protect the configuration from changes. These should be used with care, as they may require the system to be shut down to make changes when the process wouldn't otherwise warrant a shut down.

Process control systems operating under management of change procedures should also have revision tracking built into the software systems. This is especially important when multiple employees have software change rights. A revision history allows all those with change rights to monitor system software without having to trust others with similar access rights. A built in revision tracking system would allow those with change rights to prove that no changes to system software have been made. It is also a good idea to print revision history reports on a regular basis to provide additional records to prove when changes to systems software are implemented. As a secondary backup to revision histories, system software reporting tools should be used to document the system configuration. This can be used as a manual revision history record, and can be used to cross reference with the software revision tracking system to reduce the chances that security can be breached without detection.

3.2 Review and Authorization Requirements

Each change to system software must have proper authorization to proceed. As part of the documented review procedure, the control system engineer should review the proposed change to identify parts of the process control systems which might be affected by the change. Additions to the process might require controller or I/O upgrades which could affect the time required to complete the change. Depending on the control system and security options in place, the control system may have to be temporarily disabled to make process control software changes. All these consequences of change need to be communicated to others reviewing the change, as this may affect the decision to implement the change, or at the very least the timing of the implementation.

Only after all parties involved in the review process have signed off on a change and a plan for execution has been completed should changes occur. The execution plan should detail all actions necessary to implement a change, and should differentiate whether the change is permanent or only temporary. Temporary changes should have associated time limits and procedures for returning to status quo after the predetermined time period has expired. In all cases, a record keeping system needs to be used to keep up with what change was authorized and when it was implemented. These records should directly correlate with the revision tracking systems in place. All these systems will help to verify that your process control systems are in compliance with management of change procedures.

4. Training Requirements

Training employees helps them understand the nature and causes of problems arising from process operations, and increases awareness of hazards relating to work processes. An effective training program can reduce the number and severity of incidents arising from process operations, and can be critical in keeping small problems from becoming catastrophic releases. The PSM regulations require that employers provide an initial training program for any employee and/or contractor's employee involved in a regulated process, and maintain process hazard knowledge through refresher training. Employers must also maintain records certifying that employees were given and understood training information, and document the training and certification program.

4.1 Initial and Refresher Training

Automation can reduce the amount of critical information an employee must retain. As more critical tasks are handled by the automation systems, employees are required to take fewer actions upon detection of hazardous situations. But this has a downside as well, since it is also important for the operator of a hazardous process to know and understand exactly what the control system is doing, even when no operator intervention is required. Since the training should emphasize safety and health hazards as well as emergency operations, employees need to know how to operate the facility through use of the control system's display screens. An operator should be able to monitor and/or control all startup and shutdown sequences through intuitive information display, and should be able to quickly perform emergency actions based on equally informative process alarm information.

Training must also be provided to each employee involved in maintaining the on-going integrity of the process equipment, including maintenance personnel involved in the upkeep of the control system and process instrumentation. These personnel should have thorough knowledge of the system so that they can understand the difference between process problems and control system problems, and be able to effectively and quickly troubleshoot any reported difficulties. Maintenance personnel should not be allowed to make changes to the system software, but should be granted read only access to allow them to view control system diagnostic data.

Once an employee has been properly trained in a hazardous process, the employer must certify that each trained employee understood the training. This ensures that employees can effectively utilize their training to perform their work duties in a safe manner. The employer is obligated to provide refresher training to assure that the employee continues to understand and adhere to the current operating procedures of the process. Refresher training must be provided at least every three years, and more often if necessary. The actual frequency of refresher training should be based on the complexity of the process and on feedback from employees involved in the process.

4.2 Simulation Based Training

Since operators in highly automated facilities depend heavily on information provided by the control systems, it makes sense to train them in plant operations on a simulated control system identical to the online systems. A simulation based training system uses duplicate HMI equipment running duplicate process control software, with an electronically simulated process model reacting to the commands of the control systems. The simulation can be invasive or non-invasive and the method used depends on the nature of the process and the control system, the funding available, and the quality of the simulation required. In either case software is used to determine the appropriate state of process inputs to the control system based on the process control system outputs to equipment.

An invasive simulation adds code to the existing process control software to emulate the actions of process equipment. The simulation code runs in the same control processor as the process control software, which

is why it is called invasive simulation. This type of simulation requires the least amount of additional hardware and software, but can be the most difficult to implement. That is because process inputs must be simulated with native code based on process control outputs. This can be simple for discrete items like pumps and on/off valves, but can quickly become very complicated for analog process variables, for example, process vessel levels with multiple input or output flows of varying rate.

Non-invasive simulations use external hardware and software to replace the I/O structures of the unmodified process control system. This type of simulation is easier to implement due to the wide variety of vendors providing tools available, but can be more expensive initially due to the extra hardware and software required. For some control systems, process control hardware can be emulated with PC-based software, reducing the initial capital costs somewhat. Process simulation software can very quickly simulate discrete devices and can provide a very accurate simulation of analog process variables including vessel levels, process flows, temperatures, and pressures. Non-invasive simulations use a duplicate of actual process control hardware and software with the exception of the I/O structures, so the simulation tends to be more accurate. Also, non-invasive simulations are required in some other industries like pharmaceuticals for software validation purposes.

The primary benefit for using simulation based training is the ability to test an employee's knowledge in real process situations. A simulation for use in training typically does much more than model the hazardous process. It can create emergency situations to test an employee's response time and accuracy. Typically, this is done by modifying the process model for a specific piece of equipment, and allowing an instructor to create a simulated hazardous situation. A good simulation based training system tracks the operators actions, and creates training and testing reports which can be used for certification records.

5. Check Out Procedures

Now that all of your documentation is in place and your employees are fully trained, it's time to startup the facility. But before you can do that, you have to complete a pre-startup review to be sure all PSM regulations have been followed. Once you are sure you're ready, it's time to verify the mechanical integrity of the process equipment. The term mechanical integrity is a little misleading, because it is also intended to check the integrity of the process control hardware and software. Once individual component operation has been verified, you should operate the process in a test mode using water instead of raw materials and/or hazardous chemicals.

5.1 Pre-startup Safety Review

The pre-startup safety review is one last review to be sure all aspects of a hazardous process are as safe as possible before actually operating any equipment or introducing hazardous chemicals into the process. The review verifies that construction and equipment is in accordance with design specification, by review of construction drawings and documents, or by visual inspection of the process equipment, or both. All compliance documentation should be complete and up to date, reflecting all changes to process safety information, operating and maintenance procedures, as well as the emergency action plan. New facilities must have completed a process hazards analysis, and existing facilities must have followed management of change procedures to ensure all PHA information is up to date. Finally, all employees and contract employees must be adequately trained regarding the hazards and safe operation of the process, and they must be certified by the employer as being knowledgeable of all safety, operating, and emergency procedures.

After the pre-startup safety review, a control systems engineer should begin by commissioning the control system. Control system commissioning verifies that the system is in good working order in general, and the installation of the system is complete and accurate. At this time, the control processors should be

powered up, and up to date process control software loaded into the system. All data networks should be fully functional assuring the proper flow of data between various control processors, and the operator's display screens. Good software engineering practices will provide a software master control relay (MCR) to ensure that no control systems outputs are enabled during control system commissioning. The MCR should set all process control sequences to a known home state such that when the MCR is enabled, process equipment won't immediately start without operator intervention.

5.2 Mechanical Integrity Requirements

The intent of the mechanical integrity requirements are to assure that equipment used to process, store, or handle highly hazardous chemicals is designed, constructed, installed, and maintained to minimize the risk of release of such chemicals. A mechanical integrity program is different from the pre-startup safety review in that it involves the ongoing test and inspection of process equipment. Process equipment specifically covered by the PSM regulations includes pressure vessels and storage tanks, piping systems and components such as valves, relief and vent systems, pumps, emergency shutdown systems, and controls including monitoring devices, sensors, alarms, and interlocks. The elements of a mechanical integrity program include written maintenance procedures, training for process maintenance activities, and inspection and testing of covered process equipment.

Verification of the mechanical integrity of a process control system can be tedious, but will go much faster with adequate preparation. A commissioning checklist book should be prepared which outlines all tests of the control system to be performed to satisfy the requirements of the mechanical integrity program. Each test should be on a separate page detailing the test to be performed, and should include space to record the date of the test, name(s) of the people performing the test, serial number or tag-name of the equipment under test, and pass/fail results. Generally, it will take two people to test most equipment, one to physically monitor the actual equipment, and one to operate the equipment from the operator display. For example, the testing sheet for a pump would prompt to try to start the pump from the operator display to verify that it won't start because of existing interlocks. Then, either the interlocks could be made, or the pump could be placed in a manual mode and temporarily started, depending on the process application. Appropriate feedback to the operator display with verification that the pump actually ran in the proper direction would indicate a pass of the test. The pump should then be placed back into an automatic mode to reactivate interlocks.

5.3 Process Automation Commissioning

Once all process equipment has passed mechanical integrity checks, full process commissioning can be performed. Process commissioning is the act of running a simulated process without any raw materials or hazardous chemicals. Process commissioning verifies that all process components work together to perform the process activities as designed in a safe manner. Heat and pressure energy should be applied whenever possible to assure systems do not begin to leak under forces which may be present in the normally operating process. All activities necessary to complete the process commissioning should be documented as a final test step in the commissioning checklist book. The commissioning page should detail what range of process flows, temperatures, etc. are expected and provide space for pass/fail results.

Process commissioning is especially helpful where equipment is started together as a system. Start-up and shutdown routines can be verified before hazardous chemicals are introduced into the system. During this stage of testing, process control software may require minor modification to improve the safety and/or efficiency of process control. This is acceptable as long as all documentation is updated per management of change procedures before hazardous chemicals are introduced into the process. It can also be very beneficial to have actual operations personnel operating the facility during this stage. This helps build their confidence and reinforces their process knowledge, while completing the final stage of testing before placing the facility online.

6. Summary

Hopefully, this paper has provided you with a better understanding of how OSHA regulations can impact automation systems, and how they can affect your hazardous operations. Compliance documentation can be integrated into operator control displays, including process safety information, operating procedures, and emergency response. Management of change procedures suggest implementation of security to limit and track revisions to automation systems. Process simulation tools can be used to develop a fully simulated process control environment which can be used for operator training and certification. Check out procedures should proceed carefully, following OSHA guidelines for pre-startup safety review and mechanical integrity. Note that this paper was compiled to give readers an initial understanding of the subject, and that following the recommendations contained herein will not guarantee complete compliance with all OSHA regulations.

6.1 For More Information

Many thanks are extended to OSHA and the Department of Labor for providing ready access to the regulatory information used to prepare this paper. OSHA has an excellent web site which anyone with Internet access can use to continue research on the topic of PSM regulations as well as many other OSHA regulations. There, you can obtain the full text of the regulation along with documents detailing compliance guidelines, enforcement procedures, and how audits are conducted, plus much, much more. Further information can be obtained directly from OSHA or OSHA's web site at <http://www.osha.gov>.

6.2 About the Author

Mark Strube PE is a six year employee of PSI, Process Systems, Inc., a design/build engineering & construction company serving industrial clients in the corn wet milling, edible oils, specialty chemical, and food and beverage industries. Mark is a project engineer in PSI's Control Systems Group and has engineered and started up automation systems for numerous clients over the past ten years. He has a BSEE and MSEE from Memphis State University (now University of Memphis) and a Master of Engineering Management from Christian Brothers University, also in Memphis. Mark has extensive experience using control systems equipment from Allen Bradley, Rockwell Software, Modicon, Moore Products, Intellution, and Wonderware. He can be reached via e-mail at mbs@psimemphis.com, by phone at (901) 756-8250, or by US mail at 1790 Kirby Parkway, Suite 300, Memphis, TN 38138.

Impact of OSHA 1910.119 on Automation in Solvent Extraction Plants

Mark B. Strube PE



- *Presentation centers around OSHA 1910.119
- *Perspective of a controls system engineer
- *Applies to hazardous processes in general, but points out where solvent extraction facilities are impacted in particular
- *My background

Introduction to OSHA 1910.119

- Process Safety Management of Highly Hazardous Chemicals
 - Known as the PSM Rule
 - Effective Date: May 27, 1992
 - Regulates management of process safety systems to protect employees from toxic, reactive, flammable, or explosive chemicals in the workplace



The Occupational Safety and Health Administration (OSHA) as a part of the U.S. Department of Labor is in charge of making sure that employees are able to perform their job functions in the safest way possible. In **February 1992, OSHA published standard 1910.119** titled “Process Safety Management of Highly Hazardous Chemicals”. This standard **went into effect in May of 1992**, and specifically deals with the management of process safety systems which employers have to implement to protect their employees from toxic, reactive, flammable, or explosive chemicals in the workplace. Process safety systems required by the rule include detailed information about the process and the hazards of its operation, operating procedures, training requirements, startup and checkout requirements, management of change procedures, and plans for handling hazards when they occur in the workplace.

Applying the PSM Regulation

- Covers processes with more than 10,000 pounds of Highly Hazardous Chemicals
 - Applies to processes that use, store, handle, manufacture, or move HHCs on-site
 - Definition of “Process” includes all process piping and vessels, as well as any interconnected storage and day tanks
 - Hexane is considered an HHC



The Process Safety Management (PSM) standard applies to processes with more than 10,000 pounds of a flammable liquid or gas on a site in a single process. Specific chemicals listed in Appendix A to the OSHA standard have significantly lower threshold quantities (TQ). The regulation **defines a process as any activity involving a highly hazardous chemical (HHC) including any use, storage, manufacturing, handling or the on-site movement of such chemicals or a combination of these activities.** OSHA extends the boundaries of a process by including all piping and vessels which are **interconnected or are located such that a highly hazardous chemical could be involved in a potential release.**

The most common solvent used in today's solvent extraction facilities is various forms of hexane. Hexane is extremely flammable with an NFPA rating of 3, and is considered a highly hazardous chemical by OSHA regulations, but is not specifically listed in the Appendix A to the PSM regulation. Therefore, any solvent extraction facility with a process containing more than 10,000 pounds (about 1800 gallons) of hexane is required to abide by the regulations set forth by OSHA 1910.119. The threshold quantity of **10,000 pounds** need not be in the primary process equipment, but any **equipment directly piped to the primary process equipment including day tanks and storage tanks**, as these may be affected by catastrophic releases.

Exemptions from the Regulation

- Retail facilities, oil & gas drilling facilities, or normally unoccupied remote facilities
- Hydrocarbon fuels used solely for workplace consumption as a fuel
- Flammable liquids *stored* in atmospheric tanks below normal boiling point without benefit of chilling or refrigeration



Exceptions are made for sites where the threshold quantity is exceeded for hydrocarbon fuels or where the flammable liquid is stored in an atmospheric tank and kept below its normal boiling point without benefit of refrigeration or chilling. Specifically exempted from the rule are retail facilities, oil and gas drilling or servicing facilities, and normally unoccupied remote facilities. The exemptions for other types of facilities, including solvent extraction facilities, are **canceled when the process in question is located such that it may be affected by a catastrophic release in another area**. In general, storage tanks connected to the actual process equipment where the highly hazardous chemicals are used are considered part of the process, since they can be affected by catastrophic releases within the primary process vessels. In these cases, **storage tanks become part of the process, and are no longer used purely for storage, which makes the boiling point of the HHC irrelevant**. Further, this would also cause the threshold quantity of HHCs to be exceeded, making it near impossible to justify an exemption from PSM regulations.

Cancellation of Exemptions

- Connection of HHC storage vessels to primary process makes it a part of process
- HHCs must be stored below normal BP without benefit of chilling or refrigeration
- BP irrelevant to HHCs involved in process
- All HHCs must fall under an exemption or exemption is canceled



Being exempted from the requirements of the rule is not impossible but will be very difficult. In fact, it will probably be less trouble to comply with the PSM regulations than to try to avoid them. A solvent extraction facility would have an especially difficult time being exempted. It is unlikely that a modern plant could operate profitably without exceeding the threshold quantity of hexane or some other solvent. A plant would have to have **less than 10000 pounds of solvent on the site, including bulk storage, and would have to maintain records to prove that the TQ is never exceeded, even temporarily.**

Another possible exception would come under operation in a **normally unoccupied remote facility**. This can't be a separate building in the back corner of your existing property. It must be a **separate site, not close to anything, and must be able to operate without any personnel, with only periodic maintenance**. OSHA would cancel this exception for any facility which could pose a threat to any personnel or adjacent facilities if there were a catastrophic release of HHCs. Further, the expense of constructing, operating, and maintaining a "lights out" facility that would pass OSHA muster would be significantly more expensive than adhering to the regulations.

General Requirements

- Document all Process Information
- Analyze and Correct Hazards of Process
- Employee Training:
 - Operating Procedures
 - Emergency Operations
 - Certify Employee Competence
- Verify Process with Documentation



The PSM regulation **does not restrict how much or how little automation exists in your facility, but does require that you know and document exactly what that automation does.** You must train all personnel on how to operate the facility safely, what to do if something goes wrong, and be able to prove that they still know what you taught them. You must take extra care in checking out new construction, and document methods and results of your checkout procedures. You must have formal procedures for making changes to process control software, and you must be able to enforce them with appropriate security.

Talk about vagueness of regulation, show 7 page standard

- a) application
- b) definitions
- c) Employee Participation
- d) Process Safety Information
- e) Process Hazards Analysis
- f) Operating Procedures
- g) Training
- h) Contractors
- i) Pre-Startup Safety Review
- j) Mechanical Integrity
- k) Hot Work Permit
- l) Management of change
- m) incident investigation
- n) emergency planning and response
- o) compliance audits
- p) trade secrets

During an OSHA Inspection

- Program-Quality-Verification (PQV)
 - Program verifies that a program is in place
 - Quality compares program to acceptable industry practices
 - Verification determines effectiveness of safety programs
- CSHOs review documentation, interview employees, and observe site conditions to determine compliance



To pass an inspection of your PSM program, you will need to have all of your documentation in order. The inspection checks your program in three phases termed **Program-Quality-Verification (PQV)**.

Program- First, your compliance safety and health officers (CSHOs) will ask for access to all your PSM documentation to be sure you have a program in place. **Quality** - Second, the CSHO will compare the quality of the program with acceptable industry practices. Finally, the **verification** stage determines if your implementation of PSM is effective by reviewing written programs and records of activity, interviews with employees at different levels, and observation of site conditions. The CSHOs follow a **directive** published by OSHA regarding compliance guidelines and enforcement procedures. The inspection procedures outlined attempt to allow the inspector to answer yes/no questions regarding your program, with each “no” representing a citation for failure to comply. It is therefore in your best interest to have all the right documentation on hand to keep the CSHO impressed.

Failure to Comply Consequences

- Between October 1995 and September 1996
 - 112 inspections, 591 citations, \$2,792,636 fines
 - Average fine/citation was \$4,725
 - Average fine/inspection was \$25,000
 - Maximum Fines exceeded \$100,000
- Accident increases chance of inspection
- US Courts can force you to comply based on Clean Air Act of 1990



This sounds like a lot of work to satisfy a government agency. Between October 1995 and September 1996, OSHA issued 591 citations for violation of the PSM standard during 112 inspections, and levied penalties of \$2,792,636. This makes the average penalty per citation \$4,725, but since an inspection usually yields over 5 citations, the average penalty per inspection is almost \$25,000, with the maximum penalties easily reaching into six digits. Further, if you're cited, the courts can force you to comply with the regulations anyway, since the OSHA regulation is a requirement of the Clean Air Act Amendments enacted by Congress in 1990. So if you're not following the OSHA guidelines, your plant is not as safe for you or your employees as it could be. After all, safety is what OSHA is all about.

Process Safety Information

- Three basic areas of information regarding:
 - the safety and health hazards of HHCs
 - the technology of the hazardous process
 - the equipment used in the hazardous process
- Equipment documentation includes P&IDs, materials of construction, relief systems, electrical classification, and safety systems



The **basis for much of the documentation** required by the PSM regulations is the process safety information. This process safety information should be used as the basis for subsequent process hazards analysis, and is usually compiled by the technical personnel that designed the process in question. The process safety information covers three basic areas, information pertaining to the hazards of the HHCs themselves, information pertaining to the technology of the process in question, and information pertaining to the equipment used in the process. **Information about the equipment is the key area** which impacts automation systems. Equipment information required to satisfy this section of the regulation includes materials of construction, piping & instrument diagrams (P&IDs), electrical classification, relief system design and design basis, ventilation system design, and safety systems. Safety systems include many control system functions like **interlocks and detection and suppression systems**. During a PQV inspection, OSHA will request **process narrative descriptions** to satisfy this part of the review.

Process Safety Information

- Control System Safety Information
 - Equipment Interlocking
 - Safe operating limits
 - Manual override systems
 - Hazard detection and suppression
 - Hexane detection
 - HMI alarming systems
 - Alarm horns and beacons



The process narratives should explain the **safety systems and other interlocks** of the automation systems in detail adequate to conduct a process hazard analysis. **All effects on each controlled piece of equipment** should be detailed. **For example...** Note that the **override of an interlock cannot be taken casually**, and the **consequences** of overriding the interlock must be clearly documented
***talk about override security...**

hazard detection and suppression systems. This area of information centers around the control system's alarming functions. Alarms should be generated by the **most reliable equipment** and enunciated in a clear and concise fashion to all facility personnel. In PLC based systems, alarms would be generated in the PLC, while using a PC based human-machine interface HMI for monitoring and annunciation purposes only. The PC based HMI should offer an alarm display to allow an operator to see prioritized alarms throughout the facility. **Alarm horns and beacons** should also be located such that any personnel in the facility would be warned of a hazardous situation. Beacons should be **multicolored** to allow for the annunciation of simple equipment alarms, potentially hazardous early warning alarms, and emergency response alarms. The rule of thumb to remember when designing alarm systems is to **keep the operator informed about all alarms, and prioritize all alarms so that the most hazardous situations can be addresses first.**

In solvent extraction plants, **hexane detectors** should be located in key areas to detect leaks. The **process narrative should detail what action should be taken by the operator and by the control system when a release is detected.** Hazardous release detection should have a **minimum of two stages.** The first stage should give operations personnel early warning that a leak has been detected. This alarm would allow parts of the process not directly related to problem area to continue operations under direction of the automation systems while a maintenance technician checked out the leak. A second stage alarm would go into effect when hexane levels rise to hazardous levels, at which point, all automated equipment would be shut down by the control system, and the facility's emergency response plan would go into effect.

Process Hazards Analysis

- Based on Process Safety Information
- Conducted by Team Consisting of:
 - At least one employee with process specific knowledge
 - At least one person familiar with specific PHA methodology being used
 - Control System Engineer



Once the process safety information is compiled, a process hazards analysis (PHA) is conducted. The process hazards analysis should be conducted as soon as possible after process safety information is available. Existing processes at the time the PSM regulations went into effect have until May 26, 1997 to have completed all PHAs. PHAs are living documents which **must be revalidated at least every five years** to assure that the process hazard analysis is consistent with the current process. PHAs may have to be reviewed **more frequently if work processes or procedures are modified** in any way (other than replacement in kind, replacing a malfunctioning valve with a working duplicate, for example) through management of change procedures.

The PHA should be **conducted by a team** knowledgeable in engineering and process operations, with at least one employee having process specific knowledge, and one team member familiar with the specific process hazard analysis methodology being used, usually an outside consultant used for PHA facilitation. Although not specifically called for by the PSM regulation, it is a good idea to have at least one **control system engineer familiar with the process control systems** included on the PHA team. The control system engineer can answer specific questions about software interlocks and security schemes, and can explain to other team members the **consequences of failure of various aspects of the automation systems**.

The PHA should identify, evaluate, and control the hazards involved in the process. It is the **responsibility of the employer to establish a system to address the team's findings** and recommendations. All **activities initiated by the PHA should be documented** as part of the PHA and **communicated to all operating, maintenance, and other** employees whose work assignments involve the process and who may be affected by the PHA. Team findings can include any changes necessary to the process control software to minimize risks to employees.

Process Hazards Analysis

- Control Systems Engineer provides:
 - Specific knowledge of control systems in use
 - Suggested interlocking and/or security to minimize hazardous risks identified by team
 - Knowledge of human factors
 - Clarity and simplicity of HMI screens
 - Level of automation
 - Alarm display to operator



Typically, the control system engineer involved in conducting the PHA can suggest supplemental interlocking or other security schemes to minimize risks identified during the analysis of process hazards.

The analysis of the control system as it affects process hazards should address human factors including the clarity and simplicity of HMI control screens and the level of automation provided by the system. People are inconsistent by nature, and it is key to **configure the control system to provide consistent direction to operators.**

In general, always **automate simple functions** which can reduce the tasks to be performed by the operator.

Configure critical alarms to stand out from the background with a distinct color or flashing pattern, or have them take over the entire display until they are acknowledged. This will allow the operator more time to monitor critical functions, and will reduce the number of human factors which can contribute to process hazards.

Operating Procedures

- Must be consistent with Process Safety Information
- Includes information regarding:
 - Steps for each operating phase
 - Standard operating conditions and consequences of deviation
 - Safety and health considerations
 - Safety systems and their functions



The one area of compliance documentation that most directly affects automation systems is operating procedures. Operating procedures are **not a part of, nor do they replace process safety information**, but they must be **consistent** with process safety information. They must address steps for each operating phase, operating limits, safety and health considerations, and safety systems and their functions. Phases of operation to be addressed within the operating procedures include **initial startup, normal operations, temporary operations, emergency shutdowns and operations, normal shutdowns, and startups following a turnaround or emergency shutdown.**

Operating Procedures

- Steps for each Operating Phase should:
 - be automated as much as possible
 - be displayed on the operator's screen
 - keep the operator informed of current automation sequence status
 - request operator input at key points to ensure operator supervision of hazardous processes



Control systems designed for use in PSM regulated processes should include much of this **information directly on the operator's control screen**. Steps for each operating phase can be coded into the process control software and displayed on a control screen. The automation system can even help walk an operator through a startup or shutdown by laying out each specific step. **The more instrumentation that is available within a regulated process, the more the control system can do in an automated fashion**. The operator's control screen should still display all steps, but the system can automatically sequence through all steps except those which require manual intervention or confirmation by an operator. Some highly automated sequences may even request that an operator make certain entries at key points in the sequence to ensure that there is still a real person watching over the process at all times.

Two other key compliance areas include the **accessibility of operating procedures to employees** who work in or maintain a process, and the **review of the procedures** to ensure they reflect current operating practice. Accessibility is guaranteed by providing operating procedures directly within the operators control displays. However, by doing so, it makes it more difficult to maintain accuracy when process safety information changes. You can help keep online operating procedures up to date by giving small rewards to operations and maintenance staff when they discover inconsistencies. Although this should not be the primary reconciliation method, it can ensure continued accuracy, and it can also get all of your employees thinking of ways to improve the safety and efficiency of current operating procedures.

Operating Procedures

- Equipment Operating Limits
 - Standard Operating Conditions are conditions where equipment is normally operated
 - Override of SOC's
 - Allowed only with proper security
 - Should display consequences of deviation
 - Safe Operating Limits are operating conditions where safety or health hazards can occur
 - SOLs can never be overridden



Information about equipment operating limits should also be **available to the operator from the HMI displays**. There are two major types of **operating limits, discrete and analog**. Discrete operating limits define the discrete state of related equipment for operation of a pump, for example, and are **often considered interlocks**. Each piece of discrete equipment can have an associated subdisplay which specifies the discrete operating limits. This **subdisplay can offer an override function to allow operation of the equipment outside of SOC's**, but safe operating limits should never be exceeded. When operating limits are overridden, **consequences of deviation** should be displayed, and **deliberate action should be required to place equipment outside of standard operating conditions**. This action could be an extended set of keystrokes, password protection, or external keyswitch, depending on the severity of the consequences.

Analog **operating limits define the range of values** from specific instrumentation under which it is normal and safe to operate. This type of limit is more often associated with analog feedback loops traditionally controlled by single loop controllers. Today's automation systems provide the loop information but with additional detail. Loop face-plates can include discrete interlocking information as well as analog operating limits. Discrete interlocks for an analog loop might include operating state of a specific pump for a loop to operate in an automatic mode. ****Manual mode is traditionally more accessible for analog stuff**

Typically, SOC's would **define the high and low alarm setpoints**, with **safe operating limits defining the high-high and low-low alarm setpoints**. Where these limits are **dynamic**, **deviation from setpoint alarming** can be used. In any case, alarm setpoints should always be displayed for the operators use. However, **changing these setpoints must strictly follow management of change procedures**, as they are a critical part of the process safety information.

Emergency Response Planning

- Alarm systems must be:
 - distinctive for each type of alarm
 - capable of being heard over ambient noise
 - maintained in good working order
- A remote HMI node located outside of the evacuation area can provide critical process information to emergency response teams



Emergency response planning is included in the PSM standards and **defined by reference to other OSHA standards; 1910.38 - Employee Emergency Plans part (a), and 1910.120 - Hazardous Waste Operations and Emergency Response parts (a), (p), and (q).** These standards go into great detail about emergency response planning. The primary compliance issues include the existence of a **written emergency action plan**, and that **all employees are familiar** with its requirements. Of primary concerns to control systems engineers is the emergency alarm system required by reference to **OSHA standard 1910.165 - Employee Alarm Systems.**

The emergency alarm system should have several key features to comply with regulations. The alarms should be distinctive for each purpose of the alarm, which may include **multiple types such as simple equipment alarms, early warning alarms, and emergency action alarms.** The emergency alarm must be capable of being heard over ambient noise and distinctively signal the need to evacuate the work area. The emergency alarm must be maintained in operating condition and should be **checked every two months on non-supervised systems, and at least annually on supervised systems.**

For an extra level of safety in the case of a hazardous release, **consider adding a remote HMI node** in a location which can be **used to monitor actual process conditions after evacuation** of the facility. Although not required by OSHA regulations, the expense will seem very small during an emergency evacuation situation. The remote node can be used to determine the exact cause of the evacuation alarm, and can give emergency response personnel critical information to contain the hazardous release. This also provides a backup means to monitor and/or control the shutdown of automated systems from a safe location during the emergency response to a hazardous release.

Management of Change

- Changes to process or procedures require:
 - Review for impact on safety and health
 - Consideration of technical basis
 - Modification of operating procedures
 - Written execution plan to effect change
 - Training of all employees affected
 - Update of process safety information



In order to maintain a safe work environment, PSM regulations require that employers review and track all **modifications to equipment, procedures, raw materials, and processing conditions**. Any of these types of changes can affect the safety of employees working in and around the process. Minimum requirements for management of change procedures include: establishing written procedures to manage change; addressing the technical basis, impact on safety and health, modification to operating procedures, necessary time period to complete change, and authorizations required. All this information must be communicated to affected employees as the change is being implemented, and process safety information and operating procedures must be updated to reflect the new process conditions.

Each change to system software must have **proper authorization** to proceed. As part of the documented review procedure, the control system engineer should review the proposed change to **identify parts of the process control systems which might be affected by the change**. Additions to the process might require controller or I/O upgrades which could affect the time required to complete the change. Depending on the control system and security options in place, the **control system may have to be temporarily disabled** to make process control software changes. All these consequences of change need to be communicated to others reviewing the change, as this **may affect the decision to implement the change, or at the very least the timing** of the implementation.

Only after all parties involved in the review process have signed off on a change and a **plan for execution has been completed should changes occur**. The execution plan should detail all actions necessary to implement a change, and should differentiate whether the change is permanent or only temporary. Temporary changes should have associated time limits and procedures for returning to status quo after the predetermined time period has expired. In all cases, **a record keeping system needs to be used to keep up with what change was authorized and when it was implemented**. These records should directly correlate with the revision tracking systems in place. All these systems will help to verify that your process control systems are in compliance with management of change procedures.

Management of Change

- Control Systems Security
 - Password protection schemes protect process control software from unauthorized changes
 - Revision tracking tools create a history of all changes made to process control systems
 - Hard copy system documentation to provide backup to software revision tracking



In order to ensure that all changes to process control software follow management of change procedures, the control system should have **limited and secured access only**. The initial **selection of a process control system should include review of security features**. Features to look for include **password protected access and revision tracking**. Password protection systems should be set up such that maintenance personnel can troubleshoot process problems by looking at online configurations without being granted change rights. **Change rights should only be granted to a select few engineers. Passwords should be changed regularly**, and all with change right access should rotate selection of the password. This password should **never be written down** in a memo or anywhere else, and should be communicated verbally to those employees with change rights. As a backup to password protection, some control systems have **embedded switches to protect the configuration from changes**. These should be used with care, as they **may require the system to be shut down** to make changes when the process wouldn't otherwise warrant a shut down.

Process control systems operating under management of change procedures should also have **revision tracking built into the software systems**. This is especially important when multiple employees have software change rights. A revision history allows all those with change rights to **monitor system software without having to trust others** with similar access rights. A built in revision tracking system would allow those with change rights to prove that no changes to system software have been made. It is also a good idea to **print revision history reports on a regular basis** to provide additional records to prove when changes to systems software are implemented. As a secondary backup to revision histories, system software reporting tools should be used to **document the system configuration**. This **can be used as a manual revision history record**, and can be used to cross reference with the software revision tracking system to reduce the chances that security can be breached without detection.

Training Requirements

- Initial training
 - Operators should understand HMI displays
 - Maintenance techs should be able to troubleshoot control system difficulties
- Refresher training must be provided by the employer at least every 3 years
- Employees must be certified by employer that they can perform their job safely



Training employees helps them understand the nature and causes of problems arising from process operations, and increases awareness of hazards relating to work processes. An effective training program **can reduce the number and severity of incidents arising from process operations**, and can be critical in keeping small problems from becoming catastrophic releases.

Automation can reduce the amount of critical information an employee must retain. As more critical tasks are handled by the automation systems, employees are required to take fewer actions upon detection of hazardous situations. But this has a downside as well, since it is also important for the operator of a hazardous process **to know and understand exactly what the control system is doing**, even when no operator intervention is required. Since the training should emphasize safety and health hazards as well as emergency operations, **employees need to know how to operate the facility through use of the control system's display screens**. An operator should be able to monitor and/or control all startup and shutdown sequences through intuitive information display, and should be able to quickly perform emergency actions based on equally informative process alarm information.

Training must also be provided to each employee involved in maintaining the on-going integrity of the process equipment, including **maintenance personnel involved in the upkeep of the control system** and process instrumentation. These personnel should have thorough knowledge of the system so that they can **understand the difference between process problems and control system problems**, and be able to effectively and quickly troubleshoot any reported difficulties. Maintenance personnel **should not be allowed to make changes to the system** software, but should be granted read only access to allow them to view control system diagnostic data.

Once an employee has been properly trained in a hazardous process, the **employer must certify that each trained employee understood the training**. This ensures that employees can effectively utilize their training to perform their work duties in a safe manner. The employer is obligated to provide refresher training to assure that the employee continues to understand and adhere to the current operating procedures of the process. **Refresher training must be provided at least every three years**, and more often if necessary. The actual frequency of refresher training should be **based on the complexity of the process and on feedback from employees involved in the process**.

Simulation Based Training

- Emulation of process inputs to control system based on control system outputs
- Duplicate HMI node used for training can also double as emergency backup
- Allows employers to evaluate employee response to actual process situations
- Testing system can record operator actions and create certification reports



it makes sense to train them in plant operations on a simulated control system identical to the online systems. A simulation based training system uses duplicate HMI equipment running duplicate process control software, with an **electronically simulated process model** reacting to the commands of the control systems. The simulation can be invasive or non-invasive and the **method used depends on the nature of the process and the control system, the funding available, and the quality of the simulation required.**

An **invasive simulation** adds code to the existing process control software to emulate the actions of process equipment. The simulation code runs in the same control processor as the process control software, which is why it is called invasive simulation. This type of simulation **requires the least amount of additional hardware and software, but can be the most difficult to implement.**

Non-invasive simulations use external hardware and software to replace the I/O structures of the unmodified process control system. This type of simulation is **easier to implement due to the wide variety of vendors providing tools** available, but **can be more expensive initially** due to the extra hardware and software required. For some control systems, process control hardware can be emulated with PC-based software, reducing the initial capital costs somewhat. Process simulation software can very quickly simulate discrete devices and can provide a very accurate simulation of analog process variables including vessel levels, process flows, temperatures, and pressures. ***tends to be more accurate.** Also, non-invasive simulations are **required in some other industries like pharmaceuticals** for software validation purposes.

The primary benefit for using simulation based training is **the ability to test an employee's knowledge in real process situations.** A simulation for use in training typically does much more than model the hazardous process. Typically, this is done by **modifying the process model for a specific piece of equipment, and allowing an instructor to create a simulated hazardous situation.** A good simulation based training system tracks the operators actions, and creates training and testing reports which can be used for certification records.

Pre-Startup Safety Review

- Final safety review of:
 - equipment in accordance with design
 - compliance documentation up to date
 - all affected employees trained and certified
- Control System Commissioning
 - Data Networks and HMIs operational
 - Software Master Control Relay in place



The pre-startup safety review is one last review to be sure **all aspects of a hazardous process are as safe as possible before actually operating any equipment or introducing hazardous chemicals into the process.** The review verifies that **construction and equipment** is in accordance with design specification, **by review of construction drawings and documents, or by visual inspection of the process equipment, or both.** All compliance documentation should be complete and up to date, reflecting all changes to process safety information, operating and maintenance procedures, as well as the emergency action plan. New facilities must have completed a process hazards analysis, and existing facilities must have followed management of change procedures to ensure all PHA information is up to date. Finally, all employees and contract employees must be adequately trained regarding the hazards and safe operation of the process, and they must be certified by the employer as being knowledgeable of all safety, operating, and emergency procedures.

After the pre-startup safety review, a control systems engineer should begin by **commissioning the control system.** Control system commissioning **verifies that the system is in good working order** in general, and the **installation of the system is complete and accurate.** At this time, the control processors should be powered up, and up to date process control software loaded into the system. All **data networks should be fully functional** assuring the proper flow of data between various control processors, and the operator's display screens. Good software engineering practices will provide a software **master control relay (MCR)** to ensure that no control systems outputs are enabled during control system commissioning. The MCR should set all process control sequences to a known home state such that when the MCR is enabled, process equipment won't immediately start without operator intervention.

Mechanical Integrity

- Applies to integrity of all process equipment including control system and instruments
- On-going program to ensure continued proper operation of equipment
- Streamline checkout with commissioning checklist, and maintain for records
 - Detailed test procedure for specific equipment
 - Record tester name and pass/fail results



The **intent** of the mechanical integrity requirements are to assure that **equipment used to process, store, or handle highly hazardous chemicals is designed, constructed, installed, and maintained to minimize the risk of release of such chemicals**. A mechanical integrity program is different from the pre-startup safety review in that it involves the **ongoing test and inspection** of process equipment. Process equipment specifically covered by the PSM regulations includes **pressure vessels and storage tanks, piping systems and components such as valves, relief and vent systems, pumps, emergency shutdown systems, and controls including monitoring devices, sensors, alarms, and interlocks**.

The elements of a mechanical integrity program include **written maintenance procedures, training for process maintenance activities, and inspection and testing of covered process equipment**.

Verification of the mechanical integrity of a process control system can be tedious, but will go much faster with **adequate preparation**. A **commissioning checklist book** should be prepared which **outlines all tests of the control system to be performed** to satisfy the requirements of the mechanical integrity program. Each test should be on a **separate page** detailing the test to be performed, and should include **space to record the date of the test, name(s) of the people performing the test, serial number or tag-name of the equipment under test, and pass/fail results**.

Generally, it will take **two people to test** most equipment, one to physically monitor the actual equipment, and one to operate the equipment from the operator display. **For example**, the testing sheet for a pump would prompt to try to start the pump from the operator display to verify that it won't start because of existing interlocks. Then, either the interlocks could be made, or the pump could be placed in a manual mode and temporarily started, depending on the process application. Appropriate feedback to the operator display with verification that the pump actually ran in the proper direction would indicate a pass of the test. The pump should then be placed back into an automatic mode to reactivate interlocks.

Process Commissioning

- Verify equipment operation as a system before introducing hazardous chemicals
- Apply heat and pressure to verify absence of leaks and systems mechanical integrity
- Automated startup and shutdown sequences can be verified safely
- Confidence builder for operations personnel



Once all process equipment has passed mechanical integrity checks, full process commissioning can be performed. **Process commissioning is the act of running a simulated process without any raw materials or hazardous chemicals.** Process commissioning verifies that all process components work together to perform the process activities as designed in a safe manner. **Heat and pressure energy should be applied** whenever possible to assure systems do not begin to leak under forces which may be present in the normally operating process. All activities necessary to complete the process commissioning should be documented as a **final test step in the commissioning checklist book.** The commissioning page should detail **what range of process flows, temperatures, etc. are expected** and provide space for pass/fail results.

Process commissioning is especially **helpful where equipment is started together as a system.** **Start-up and shutdown routines can be verified** before hazardous chemicals are introduced into the system. During this stage of testing, process control software may require minor modification to improve the safety and/or efficiency of process control. This is acceptable as long as all documentation is updated per management of change procedures before hazardous chemicals are introduced into the process. It can also be very **beneficial to have actual operations personnel operating the facility during this stage.** This helps build their confidence and reinforces their process knowledge, while completing the final stage of testing before placing the facility online.

Impact on Automation Systems

- Compliance documentation integrated into control system operator displays
- Security procedures to protect control system from unauthorized changes
- Process simulation tools for training, certification, and checkout
- Detailed checkout procedures



Hopefully, this paper has provided you with a better understanding of how OSHA regulations can impact automation systems, and how they can affect your hazardous operations. Compliance documentation can be integrated into operator control displays, including process safety information, operating procedures, and emergency response. Management of change procedures suggest implementation of security to limit and track revisions to automation systems. Process simulation tools can be used to develop a fully simulated process control environment which can be used for operator training and certification. Check out procedures should proceed carefully, following OSHA guidelines for pre-startup safety review and mechanical integrity. **Note that this paper was compiled to give readers an initial understanding of the subject, and that following the recommendations contained herein will not guarantee complete compliance with all OSHA regulations.**

<http://www.osha.gov>.

Bioengineering of Oilseeds

Frank Orthoefer

Monsanto Co.

St. Louis, MO



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

 *Optimizing Tocopherol Value
in Deodorizer Distillate*

Leo Walsh
Henkel Corporation
LaGrange, IL



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

OPTIMIZING TOCOPHEROL VALUE IN DEODORIZER DISTILLATE

Leo Walsh, Henkel Corporation, Fine Chemicals Division
5325 South 9th Avenue, LaGrange, IL, 60525-3602

Introduction

This article is a guide for vegetable oil refiners to increase the value of their deodorizer distillate. The recommendations in this article are designed to have the optimum effect on increasing the tocopherol value of deodorizer distillate without negatively impacting the quality of the oil being refined.

Deodorization is a steam stripping process which is performed under vacuum at high temperature. It is the final step of vegetable oil refining and produces a bland stable oil. Deodorizer distillate is a by-product of the deodorization process. Deodorizer distillate contains a host of compounds including free fatty acids, mono- and di-glycerides, odoriferous compounds, oxidation by-products, tocopherols, sterols, and pesticides.

Historically, deodorizer distillate was used as an industrial waste oil or burned for its BTU value. At one time deodorizer distillate was used as a caloric supplement in animal feed in the United States. Deodorizer distillate is not recommended for this use today because of the likelihood of pesticide contamination. Deodorizer distillate can still be used as an industrial waste oil or burned. However deodorizer distillate, containing a significant percentage of tocopherols, is more valuable for its tocopherol content.

Tocopherols are natural occurring anti-oxidants in vegetable oils and are the source of Vitamin E in the diet. Natural Vitamin E, which is optically pure, is more biologically active on a per weight basis than synthetic Vitamin E, which is a mixture of stereoisomers. Tocopherols are concentrated in deodorizer distillate during the deodorization step. As a result, deodorizer distillate is a good source for natural tocopherols which are used to make natural Vitamin E.

Deodorizer distillate is also a good source for phytosterols. Historically phytosterols were used as precursors for cortico steroids. Today there is increasing interest in phytosterols because of the potential ability of certain phytosterols to inhibit the absorption of cholesterol. It is important to note that, although there is increasing interest in phytosterols, the value of phytosterols today is still significantly less than tocopherols. The value of deodorizer distillate, therefore, is essentially based on the tocopherol content.

Figure 1 shows the relationship between the percentage of tocopherol in deodorizer distillate and the current value of distillate in U.S. dollars per ton. It also shows the current alternative value for distillate (BTU value, industrial oil value, etc.).

(insert Fig. 1)

The value of tocopherol is a function of supply and demand and has fluctuated over time. Figure 2 shows the historical value of tocopherol.

(insert Fig. 2)

In 1993 several significant human studies were published that indicated the health benefits of taking natural Vitamin E. As a result, the demand for Natural Vitamin E began rising. In early 1996 there was a temporary shortage of distillate as new manufacturers of natural Vitamin E began stockpiling their captive distillate. This temporary shortage raised the price of distillate to a historical high level. By late 1996 the supply of distillate exceeded the demand as new sources were developed.

Today the demand for natural Vitamin E exceeds supply because the increases in production capacity have lagged the demand. With new natural Vitamin E manufacturers entering the market, there should be more than enough production capacity to meet future

demand. The challenge will be to develop and optimize new sources for deodorizer distillate as natural Vitamin E production increases.

Discussion

There are three basic requirements for a vegetable oil refiner to capture tocopherol value from deodorizer distillate. The oil being refined must be deodorized. The oil being deodorized must contain tocopherol. And, the deodorizer distillate must be condensed and collected.

It would be difficult to justify the purchase of a deodorizer or a change in the type of oil being refined solely on the basis of capturing tocopherol value. A scrubber is used in vegetable oil refining to condense deodorizer distillate. A scrubber is a contact condenser that uses a small amount of cooled oil to condense components volatilized in the deodorizer. The purchase of a scrubber can normally be justified on the basis of capturing tocopherol value.

The type of oil being refined has a profound effect on the tocopherol content of the deodorizer distillate. Some crude oils contain significantly higher percentages of tocopherols than others. Figure 3 shows the relative levels of tocopherol in various crude oils
(insert Fig. 3)

Of the major vegetable oils refined in the world, crude soybean oil contains the highest levels of tocopherols, followed by corn, cotton, sunflower, rapeseed and peanut oils.

Olive oil contains very low levels of tocopherols. Olive oil distillate is not considered to be a good source for tocopherols at this time. However, as the demand for natural Vitamin E increases and the availability of tocopherol containing distillates decrease, olive oil distillate may be sought after in the future.

Deodorizer distillate obtained under good conditions can vary solely on the crude oil source. Table 1 shows typical tocopherol levels in deodorizer distillate obtained from crude oils under good operating conditions. Soybean oil distillates have historically contained the highest levels of tocopherols.

(insert Table 1)

Although palm oil contains tocopherols, it also contains high levels of tocotrienols. Tocotrienols can not be economically separated from tocopherols nor can they be economically converted into racemically pure tocopherols. As a result, palm oil distillate is undesirable as a source of tocopherols.

Oils from animal sources, such as tallow, lard and fish oils, have negligible levels of tocopherols. In addition to being undesirable for their tocopherol content, animal oil distillates and mixtures of animal and vegetable oil distillate are prohibited from Kosher certification. Kosher approval for food ingredients, such as antioxidants, is a requirement of most large food manufacturers in the United States.

Conditions Impacting Tocopherol Yields

There are three areas where vegetable oil refiners can focus their efforts to have the greatest impact on increasing the value of deodorizer distillate. The three areas include 1) optimizing the deodorizer and scrubber conditions to recover tocopherols, 2) preserving the tocopherols once they have been recovered , and 3) minimizing the dilution of tocopherols during processing and storage.

1) Optimizing Deodorizer and Scrubber Conditions

The operating conditions which have the greatest impact on tocopherol levels in deodorizer distillate are deodorization time (flow rate, metering rate, residence time), deodorization temperature, scrubber temperature, steam (rate, weight and volume) and vacuum.

(insert Fig. 4)

It is unlikely that a vegetable oil refiner would make significant changes in the rate or residence time of the oil in the deodorizer for the sole purpose of capturing tocopherol value. A vegetable oil refiner with limited capacity is more concerned with maximizing the throughput of oil through their plant. However, in situations where there is clearly an excess in capacity, deodorization times can be increased to the point where oil loss, oil quality and energy cost are balanced with the additional value obtained from increasing tocopherol levels.

The conditions which can be optimized with a minimal impact on oil production and minimal impact on the finished oil quality are deodorization temperature, scrubber temperature and steam rate.

General rules of thumb:

The typical amount of deodorizer distillate recovered from refined oils is between 0.2% and 0.3% based on the weight of oil being deodorized. Values as high as 0.45% have been successfully recovered.

The most important variable in deodorization and tocopherol recovery is deodorization temperature. Temperature is important because of its effect on the vapor pressure of volatile components. Deodorization temperatures can typically range between 240°C and 275°C (460°F

and 530°F.) For optimal tocopherol recovery, temperatures should be above 260°C (502°F).

At these temperatures the quality of the finished oil is not significantly affected.

Recently, vegetable oil refiners have become concerned with the formation of trans-fatty acids. Although, there is some formation of trans-fatty acids during deodorization it is minor compared to the formation of trans-fatty acids during the hydrogenation process.

Scrubber temperatures are best maintained as low as possible while maintaining the handling characteristics of the recycled material. In general, temperatures between 57°C and 63°C are used for normal oil stocks and product blends in the United States.

Typically scrubber temperatures are related to the Iodine Value (I.V.) of the oil. A scrubber can be maintained at lower temperatures when the stock oil has a high Iodine Value. Vegetable oil distillates with high levels of phytosterols may plug the heat exchanger of the scrubber if the circulating temperature is too low. Temperatures as low as 45°C can be used for distillates from oils having high I.V.'s or where there is no plugging problems due to phytosterols.

Steam acts as a carrier to remove volatile components from the oil being deodorized. Steam is also used to create turbulence which intimately mixes the steam with the oil. Excessive steam can cause misting which can lead to neutral oil carry-over or splashing which can lead to neutral oil loss as shell drain. Ideally the steam rate should create as much turbulence as possible to create maximum mixing, but should be low enough to prevent significant splashing and carry-over. In general a good steam rate is between 0.5 and 4.0% based on the weight of oil being processed.

It is important to remember that steam volume is more important than steam weight in deodorization. With a given weight of steam the volume becomes greater as the pressure decreases. A good vacuum precludes the need for a large weight of steam. In general, 5 mm Hg is a good vacuum for deodorization.

Most vegetable oil refiners do not have the ability to control the level of vacuum. With a properly designed and operating system, vacuums are typically 5 mm Hg or less. Air leaks, faulty equipment or excessive amounts of steam can lead to insufficient vacuum. If a system is being operated as designed and pressures are significantly higher than 5 mm Hg an additional vacuum stage may be justified by both reduced steam costs and increased tocopherol production.

2) Preserving Tocopherols

Tocopherols collected under the best conditions can be lost if the distillate is not stored properly. Tocopherols are anti-oxidants. They can degrade or be destroyed by exposure to oxygen, heat, moisture and the by-products of metal corrosion. Storing deodorizer distillate properly becomes more important the longer the distillate is being stored. Proper storage tanks can usually be justified based on the value captured from distillate sales.

The best conditions for storing distillate include storage at ambient temperatures, storage under a nitrogen blanket, temporary gentle heating for handling purposes and a storage tank constructed of a noncorroding material such as stainless steel.

The worst conditions for storing deodorizer distillate include storage in an area where the tank is exposed to heat or direct sunlight, storage without an inert gas blanket, storage in a tank constructed of carbon steel, storing distillate for periods longer than 3 months and storing

distillate with moisture levels greater than 3%. Moisture in a storage tank should be minimized. One way to limit moisture is to use nitrogen instead of steam to blow out inlet lines.

(insert Fig. 5)

Monitoring the ratio of tocopherols to total sterols (after saponification) is a good way to determine if degradation is occurring during processing or storage. Tocopherols will degrade under conditions that sterols will not. If the percentage of total sterols relative to tocopherols is large or if it is increasing, degradation is occurring.

(insert Table 2)

3) Minimizing Dilution

The value of deodorizer distillate sold for its tocopherol content is based primarily on the amount of tocopherol in the distillate. Any components that dilute the tocopherol content in the distillate also lowers the value of the distillate.

Tocopherols can be diluted during processing in a number of ways. Physical refining carries free fatty acids into deodorizer distillate. These fatty acids would otherwise be removed as soapstock in a chemical refining process. Hydrogenation generates free fatty acids in vegetable oils. These free fatty acids are also carried over into the distillate.

It is unlikely that a vegetable oil refiner would decide to change from physical refining to chemical refining based solely on capturing the value of tocopherol. It is also unlikely that a refiner would stop hydrogenating for the same reason. However, there are other factors that contribute to the dilution of tocopherols which can be avoided. Neutral oil carry-over, the addition of shell drain to distillate and the addition of hot well skimmings to distillate all contribute to the dilution of tocopherols and can all be avoided.

Minimizing neutral oil loss to deodorizer distillate not only increases the value of the distillate but improves the yield of refined oil. Neutral oil can be carried over into distillate when fine droplets of atomized oil are carried into the scrubber. Oil atomization can occur with excessive steam. It can also occur when the demister pad in the deodorizer is improperly aligned above the tray.

Monitoring the ratio of Acid Value to Saponification Value is a good way of determining whether or not there is excessive neutral oil carry over in deodorizer distillate. Typically the ratio of Acid Value to Saponification Value should be 0.67 or greater. If this ratio is significantly less than 0.67 steps should be taken to identify where the excess neutral oil is originating.

Monitoring the percentage of Unsaponifiables and Saponifiables is another way to determine if there is excessive neutral oil in deodorizer distillate. Saponifiables include free fatty acids and neutral oil. Unsaponifiables include sterols and tocopherols. Typically deodorizer distillate contains 35% unsaponifiables. Excessive neutral oil carryover can dilute this to lower levels.

Excessive amounts of oil in the shell drain can occur when steam splashes oil out of a tray and into the shell of the deodorizer. It can also occur when a tray or demister pad is misaligned. Shell drain is almost always neutral oil which is very low in tocopherols. For this reason shell drain will dilute the tocopherol concentration and should not be added to the deodorizer distillate.

Shell drain can also form when vapors condense on cold spots in the deodorizer. A tocopherol analysis of the shell drain will indicate if this is occurring. If the analysis shows

significantly more than 1% tocopherol there is more than likely a cold spot in the deodorizer condensing vapors.

Excessive amounts of hot well skimmings indicate that the scrubber is not operating properly. This can occur when the demister pad in the scrubber is misaligned or when the cooling system or contact system of the scrubber is not working properly. Tocopherols in hot well skimmings usually degrade due to the exposure to oxygen and water. Because of this degradation, hot well skimmings have a very low percentage of tocopherol. In addition to low tocopherols, hot well skimmings usually contain emulsified water. As a result, hot well skimmings not only dilute the tocopherol content of the deodorizer distillate, but the water in the skimmings can destroy the tocopherols. For this reason hot well skimmings should not be added to deodorizer distillate.

Finally, this article has outlined ways of obtaining optimal levels of tocopherols in deodorizer distillate. Manufacturers of natural Vitamin E have paid a premium for deodorizer distillate containing high concentrations of tocopherols. This is one reason why it makes economic sense for vegetable oil manufacturers to optimize their tocopherol yields. Some vegetable oil manufacturers and entrepreneurs have considered purchasing equipment to further concentrate tocopherols after the distillate has been collected. Before investing in these technologies it is important to understand the economic constraints of concentration.

When distillate is shipped internationally the cost of ocean freight can be significantly greater than typical domestic freight costs. It costs more per pound to ship distillate with 5% tocopherol than it does to ship an equivalent weight with 10% tocopherol. Manufacturers of

natural Vitamin E realize this and pay a percentage of the freight savings as a premium to motivate vegetable oil manufacturers to obtain higher concentrations.

This indicates the maximum value that can be extracted from concentration is the cost difference between shipping distillate at the starting concentration and shipping distillate at the final concentration. Consequently, a higher premium will be paid for concentrating from 4% to 15% than for concentrating from 15% to 30%.

In most cases if a vegetable oil manufacturer wants to increase their tocopherol levels from 4% to 15% it makes more economic sense to optimize their deodorization process than it does to invest in concentration equipment.

Another economic constraint of concentration, that is of particular interest to manufacturers of natural Vitamin E, is yield. The value of the tocopherol lost during concentration will be greater than the premium paid for concentrating unless the percent yield is in the high nineties. In most cases having more tocopherol is more important than having concentrated tocopherol.

Figure 1.

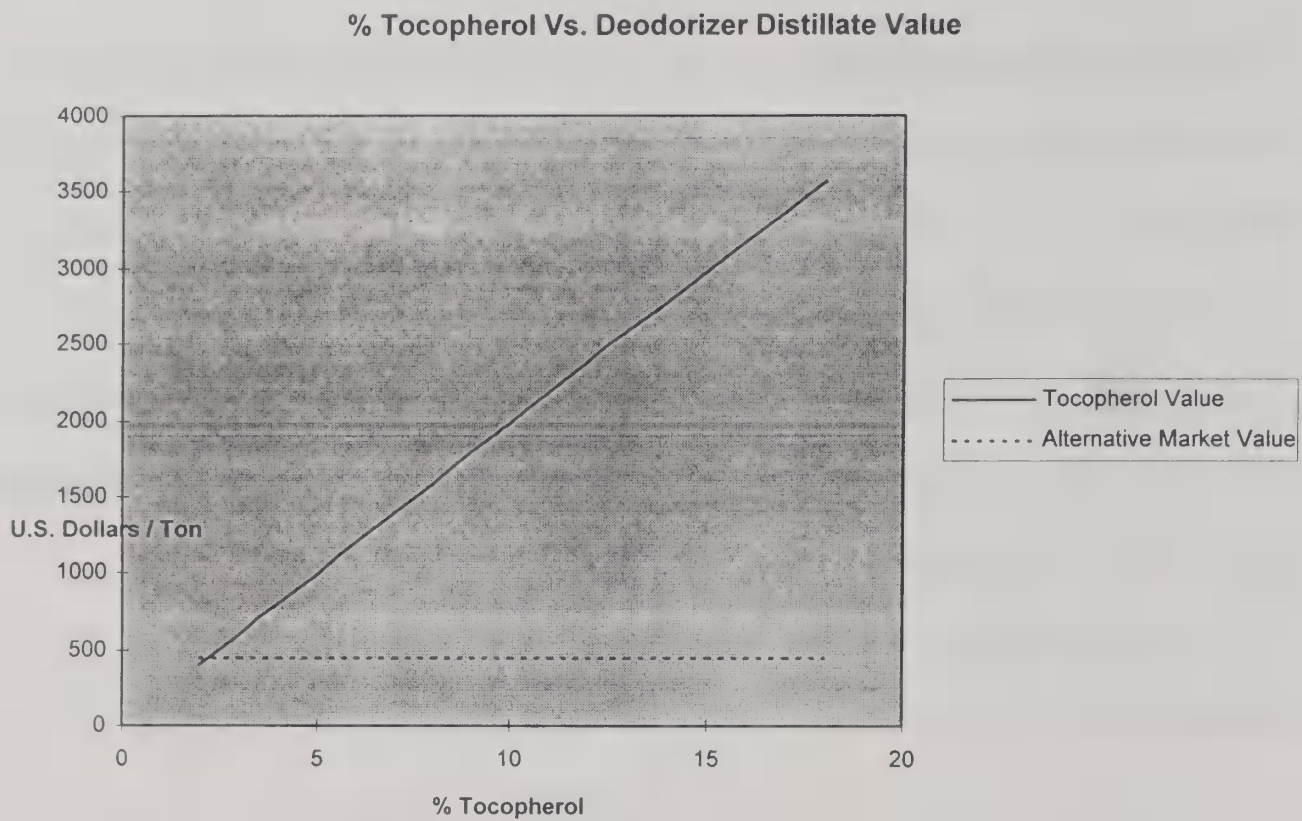


Figure 2.

Distillate Price \$/lb Tocopherol

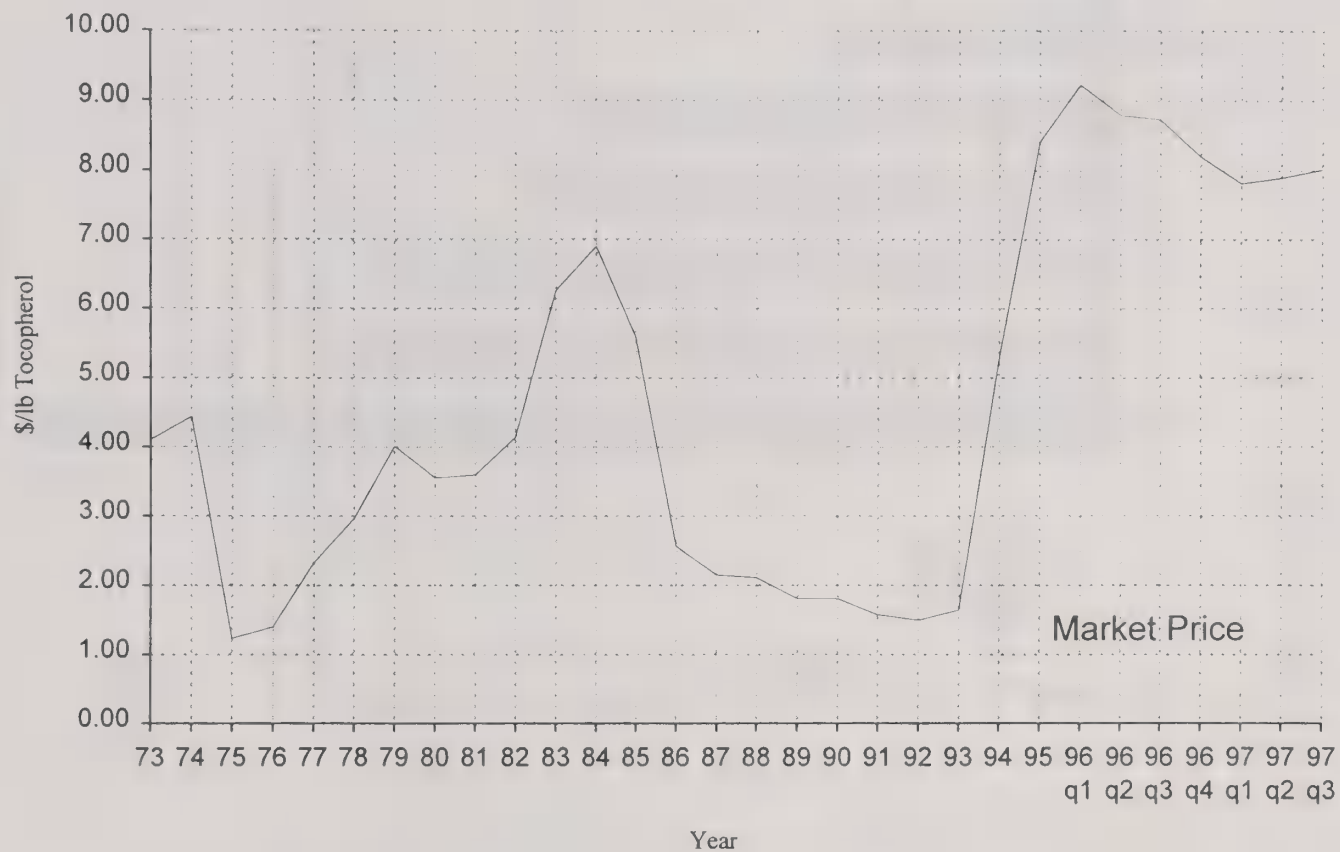


Figure 3.

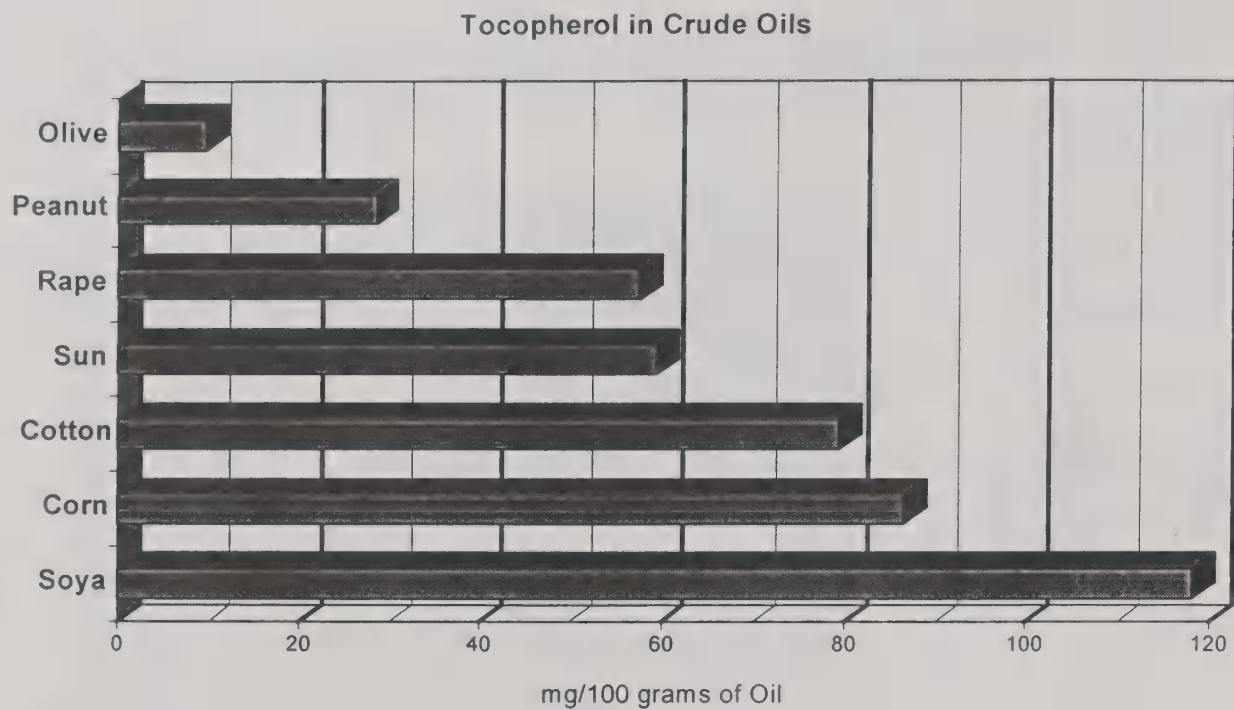


Table 1. Tocopherol Content in Vegetable Oil Deodorizer Distillate

Oil Type	% Tocopherol
Soya	10 - 14%
Corn	7 - 10%
Cotton	6 - 10%
Sun	5 - 8%
Rapeseed	4 - 7%
Peanut	2 - 5%

Figure 4. Diagram of Deodorization System

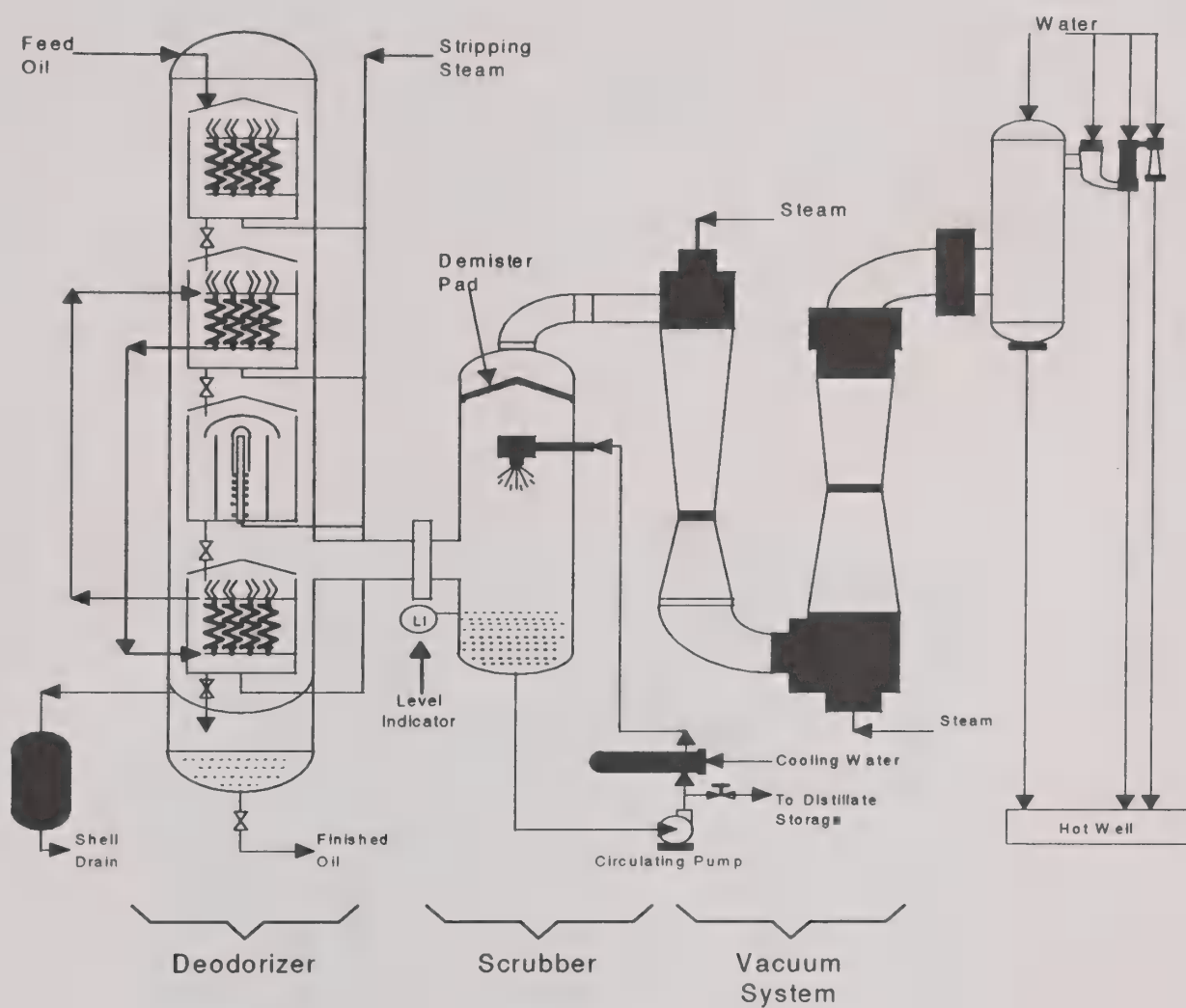


Figure 5. Diagram of Deodorizer Distillate Storage System

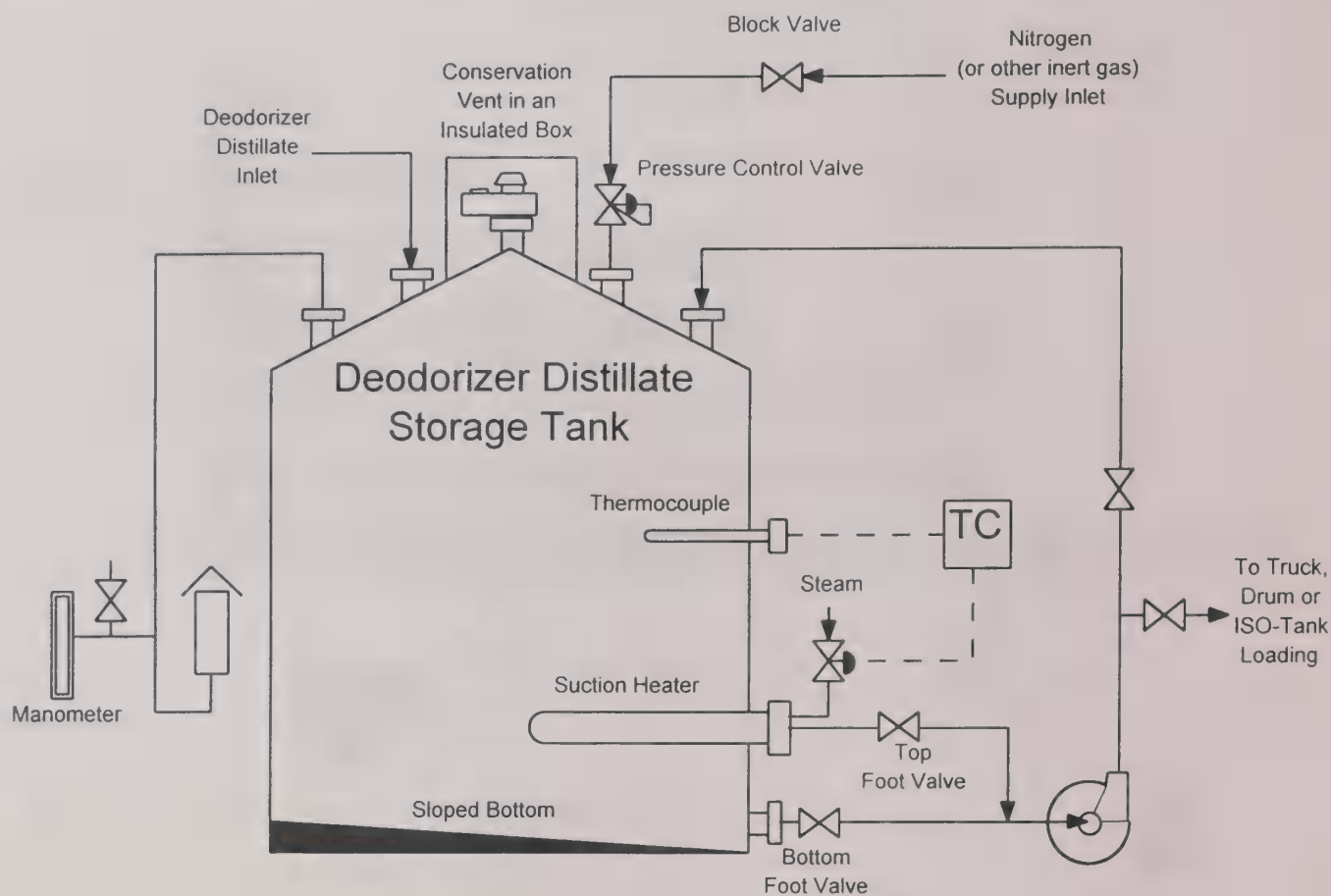


Table 2. Vegetable Oil Sterol : Tocopherol Ratio

Distillate	Sterol : Tocopherol Ratio
Soya	1.3 : 1.0
Rapeseed	1.6 : 1.0
Sunflower	2.4 : 1.0
Corn	2.7 : 1.0
Cottonseed	3.1 : 1.0
Peanut	4.0 : 1.0

Maximizing Profits by Minimizing Energy

Robert Stroup
French Oil Mill Machinery Co.
Piqua, OH



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

MAXIMIZING PROFITS

BY

MINIMIZING ENERGY

OILSEED CONFERENCE

Doubletree Hotel

New Orleans, LA

March 8-10, 1998

by

Robert L. Stroup

Managing Director, Oilseed Division

The French Oil Mill Machinery Company

Piqua, Ohio, U.S.A.

MAXIMIZING PROFITS BY MINIMIZING ENERGY

For the oilseed processing industry, energy continues to be the second largest manufacturing cost after labor. In a study by the USDA of six cooperative cottonseed plants, the following average manufacturing costs were reported:

<u>ITEM</u>	<u>COST/TON</u>	<u>TOTAL</u>
Labor	\$12.44	34.5%
Energy	7.42	20.6%
Repairs	5.65	15.7%
Depreciation	4.56	12.7%
Other	<u>5.95</u>	<u>16.5%</u>
Total	\$36.02	100.0%

TABLE 1

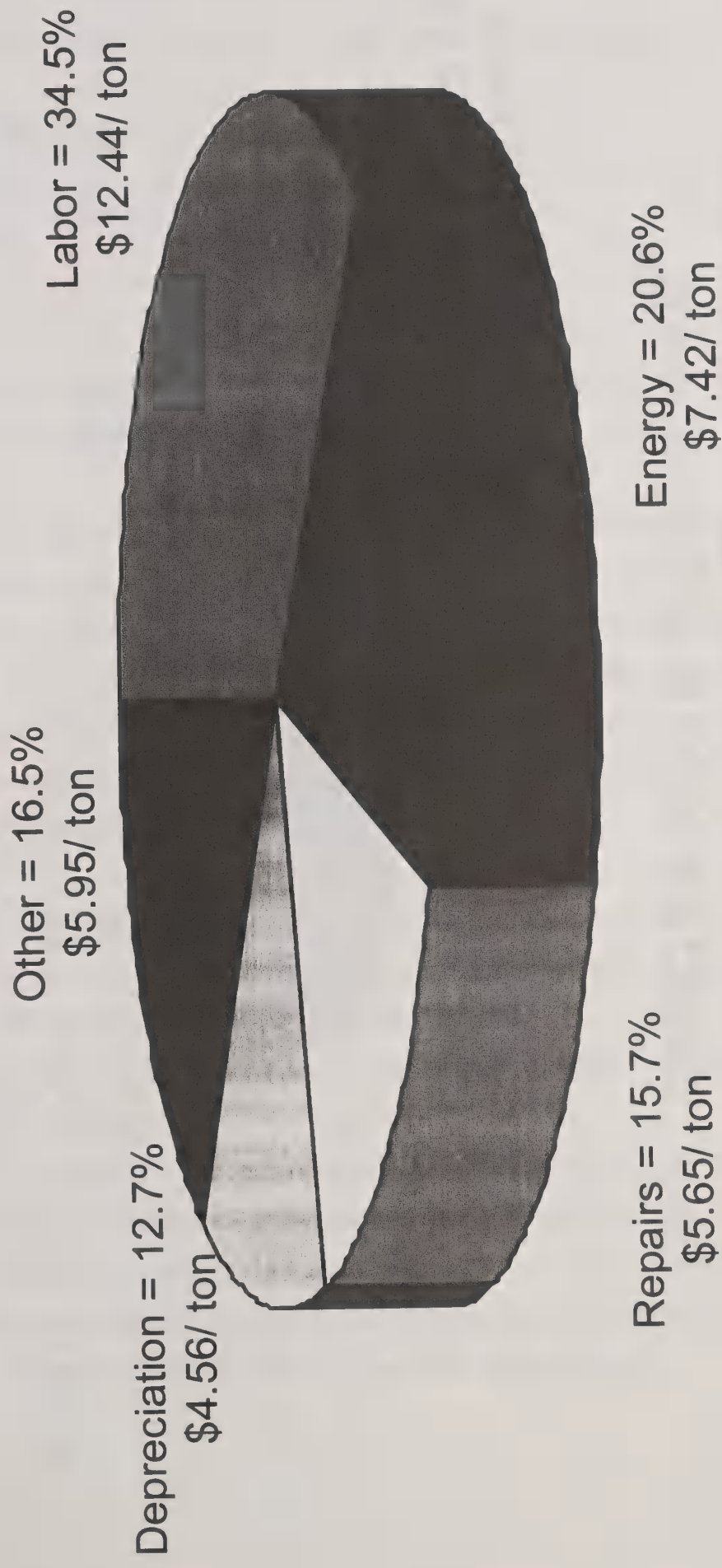
Chart 1 shows the energy and total costs graphically for the six cottonseed plants studied.

Chart 2 shows the energy and total costs for a typical soybean plant. Whether you are crushing cottonseeds, soybeans, or in fact, any oilseed, energy is a cost that can and must be managed in order to maximize profits.

In the cottonseed plants evaluated, manufacturing, administrative, and financial costs were studied. Interest and exchange costs averaged \$3.47/ton, while energy costs averaged more than twice that: \$7.44/ton.

In your own individual plants and offices, are you committing the necessary resources to manage energy?

Average Manufacturing Costs



Source: USDA

Chart 1



Cost of Energy

Oilseed Crushing Process

Based on 1200 MTPD Soybeans

ELECTRICITY - 1455 kwh x
\$.06/kwh x 8400 hrs/yr = \$733,320



STEAM - 30,121 LBS/hr x \$4.00/1000 lbs x
8400 hrs/hr = \$1,012,065

NOTE: Processing Energy only -
for Preparation and Extraction

Chart 2

In a typical processing business environment, you need to look at T.O.P. - the three primary areas where good Energy Management can pay off:

Transportation

Office

Plant

TRANSPORTATION ENERGY

In the Transportation area, the three most important words are Maintenance - Maintenance - Scheduling.

The best Energy Management plan for transportation is a well-managed maintenance program for vehicles and lift trucks. Fuel, in the form of gasoline, diesel, and propane, is consumed every day by trucks, company cars, and lift trucks. A well-designed maintenance program that optimizes fuel efficiency will help maximize profits. However, the savings generated by maintaining vehicles properly in one month can disappear in one day if vehicle use is not scheduled and managed. Consider these questions:

1. Is a local delivery service cheaper than pick up by you?
2. Can you replace gasoline or electric lift trucks with propane or CNG (compressed natural gas) units?
3. Is renting a bus cheaper and more fuel efficient than a caravan of cars or pickups?
4. Is it better to rent one way and fly home rather than drive round trip?
5. Would diesel CNG power lower your fuel costs and total operating costs compared to gasoline?
6. Is every effort being made to combine truck trips and carry full loads?
7. Are you selecting the best routes for fuel efficiency?

8. Do you have a volume “deal” on gasoline natural and diesel? If not, Shell wants to talk.
9. Are you using the fuel record and management services of the large oil companies?
10. Are you using fuel-efficient vehicles for local transportation where comfort is not a factor; or are you using a 1-ton Stake Body for ½-ton pickup job?
11. Does one person have the overall responsibility to manage or coordinate your total vehicle program?
12. Do you monitor vehicle energy performance? Tune-up or replace when performance lags?

Attention to the “T” can contribute to energy savings, which translates into more profits.

OFFICE ENERGY

The big “O” or office is the next area that benefits from Energy Management. One might say that compared to the plant, the energy used by the office is insignificant. But, save a kilowatt here and a kilowatt there, and you could be looking at megawatts over a year’s time. Even more important, management must **lead by example**. If energy management is good for the plant, then it has to be good for the office. To paraphrase an old saying:

“Energy Management for the plant begins in the office!”

Office facilities offer many opportunities for conservation, including HVAC systems, hot water, lighting, and exit lights, just to name a few.

HVAC

The Heating Ventilating Air Conditioning System (HVAC) is the largest energy consumer in most offices. The easiest way to cut HVAC costs is to maintain the equipment on a regular basis.

Develop a regular check and maintenance program for your office heating equipment and you could save as much as 5% in energy costs.

Combustion Efficiency

The flue-gas composition of a boiler or furnace is frequently overlooked. It should be checked at least twice during the heating season.

The ideal flue gas composition in the flue is shown in Table 2.

FLUE GAS COMPOSITION	
CO ₂ %	
Gas Fired Unit:	9% - 10%
Oil Fired - #2	11.5% - 12.8%
Oil Fired - #6	13% - 13.8%

TABLE 2

A check list of maintenance areas for heating equipment is shown in Chart 3.

Maintenance Checklist for Gas and Oil Heating Equipment

- Check flue gas composition
- Examine flue
- Measure exhaust gas temperature
- Clean heat exchanger surfaces often
- Clean water handling equipment to eliminate lime and scale build-up
- Keep furnace or boiler tubes free of soot, sludge, or fly ash
- Replace automatic blowdown cycle on boilers as needed



Maintenance Checklist for Gas and Oil Heating Equipment cont.

- Maintain insulation on boiler
- Calibrate controls frequently
- Check for water leaks
- Check for air leaks in combustion chamber
- Periodically check fuel oil temperature
- Keep economizers and preheaters clean and working
- Keep fan lubricated per manufacturer's recommendations
- Clean fan blades
- Check fan rotation and alignment

ELECTRIC HEATERS

Electric heaters and infrared heaters are more costly to operate, but easier to maintain. Some offices think they are maintenance-free. Not true. See Chart 4 for the electric heater maintenance checklist.

HOTWATER HEAT

Many offices, particularly in the North, use a hot water boiler for heating. You can save energy (gas or oil) by installing an outside air reset control. These controls are simple, inexpensive, and typically pay for themselves in one year.

Here is how a typical hot water heating system is controlled:

- Hot water boilers are designed to provide heating water at temperature of 180° F.
- A sensor turns burner on when the water temperature drops below 170° F.
- A sensor turns burner off when temperature reaches 180° F.

During cold weather (0° to 20° F.) 180° F water is satisfactory. During mild weather, 30° F to 60°, 180° F water will tend to overheat your office.

An outside air reset control reduces the maximum boiler water temperature depending on the outside temperature. For example, at 0° F, 180° F water is fine; but at 40° F., the heating water only needs to be 130° F.

Maintenance Check for Electric Heaters

- Calibrate controls often
- Check heat transfer surfaces for obstructions and keep clean
- Check fan rotation and alignment
- Keep fan lubricated per manufacturer's recommendations
- Clean fan blades
- Infrared heaters - check and adjust beam
- Dust reflectors

Also, by ***automatically resetting the hot water temperature during mild weather, you may save on air conditioning costs.*** How many times have you been in an office heated with hot water with the air conditioning on and the temperature outside is 50° F?

Hot Water

Your maintenance people might tell you that the hot water cannot be reset because the hot water boiler has a heat exchanger for bathroom, canteen, or maintenance sink hot water. If you do, you are using an “Energy Waster” for domestic-type hot water! If you have a single 55 or 80 gallon electric or gas hot water heater serving all of your hot water needs you are probably wasting energy.

A better solution might be to install a single 10 or 12 gallon electric hot water heater to service each bathroom complex or canteen complex. This will save energy by reducing losses at the heater by at least 60% (less surface area). Also, the radiation losses of the 1/2” or 3/4” copper tubing running from the 55 or 80 gallon central hot water heaters can be substantial - a 100-foot distribution line of 3/4” copper tubing has almost 100% more surface for radiating heat loss than a 10 or 12 gallon water heater. No wonder some bathrooms never have warm water!

Outside Air

Outside air can be your cheapest source of air conditioning. However, many builders and HVAC engineers neglect to automatically control the fresh air intake to the HVAC system. The principle is simple: When the outside temperature is lower than 70° F., a free source of cool air is available (assuming dehumidification needs are met). The HVAC system needs to bring in outside air anyway for air exchange comfort and health reasons, so why not control the percent of outside air automatically depending on outside temperature? This can dramatically lower your air conditioning costs at least 5-10% in mild weather.

Ceiling Fans

The old-fashioned ceiling fan can lower both your heating and air conditioning costs, particularly in areas with high ceilings. They work on the simple principle that ***“hot air rises and cold air falls.”*** Moving air also feels cooler since there is more cooling by evaporation from the skin.

Ceiling fans are well accepted in the home, church, gymnasium, etc. However, many offices seem to overlook their energy-saving and comfort advantages. Several 50" or 60" fans strategically placed in an office can allow you to raise the room temperature at least 2-3 degrees, thereby lowering your cooling energy cost for air conditioning about 20% when it is 82° F outside and 10% when it is 92° F outside (based on 72° F without fans and 74° F with fans). Besides saving energy, people are more comfortable.

OFFICE LIGHTING

Day Lighting

A very visible place for managing energy in the office is lighting. The best and cheapest source of light is sunlight, particularly in the wintertime. Designing for natural lighting in the summertime requires more attention to the sun's effect on the air conditioning system's heat load. Therefore, blinds, overhangs, north-facing windows, tinted glass, sky light position, etc., must all be carefully considered.

Sky lights are frequently used in the home, but often forgotten in the office, warehouse, or plant. Wal-Mart recently determined that they had a 3-year payback on the cost of installing skylights with automatic controls to turn off lamps when the skylight provided adequate lighting.

Did you ever notice the old buildings in the North with “saw-tooth” roofs that incorporated vertical skylights on one side? Your grandfathers or great-grandfathers knew that natural light was free. However, these old “saw-tooth” roofs were also a source of heat loss. Many of them, therefore, have been “boarded up” with insulation and painted - a quick fix, but an increase in lighting costs. A better solution for the long-term overall system energy savings would be to replace the single pane windows with double or triple panes. This provides good lighting plus insulation from heat loss. Special low “E” glazing will allow visible light to enter the building while excluding a good portion of the IR (heat) and UV light.

Fluorescent Lighting

Most offices have fluorescent lighting. This represents a real opportunity for energy savings, better light, and a chance to be friendly to the environment.

The opportunity lies in replacing old style T12 lamps with magnetic ballasts containing PCB's with T8 lamps using electronic ballasts. Electronic ballasts are not only more energy efficient, but also give a better light based on the CRI Color Index used by lighting engineers - especially important for merchandising and task lighting.

The old standard lamps which you will probably find in your 4-foot fixtures are F40T12 where:

F = fluorescent

40 = watts

T = tube

12 = diameter of lamp in 1/8th increments, or 1.5 inches

Most older T12 lamps use a magnetic ballast - the least efficient ballast. The function of a ballast is to provide a high starting wattage followed by a current-limiting function. These magnetic ballasts required one ballast for two lamps and used 10-12 watts just for the ballast.

T8

The latest and most energy efficient fluorescent lamp available today is the F32T8 lamp. It is a 1.0" lamp and uses an electronic ballast. The T-8 [for style, T8 or T-8, T12 or T-12?] lamps incorporate more costly rare earth phosphors to coat the lamp. This results in more efficient light output compared to the old style T-12 lamps. The smaller diameter lamp also ***allows the same usable light to exit the fixture and save 20-40% of energy.***

Chart 5 shows the savings in operating costs in a 10,000-square-foot building. The T-8 lamp delivers more lumens/watt and maintains a higher light output over its life than a T-12.

If the magnetic ballasts in your office are near the end of their rated life (15-20 years), it is soon time to replace them with electronic ballasts. T-12 lamps also can use electronic ballasts (different electronic ballasts than for T8) but why not use the T-8 lamp with its greater efficiency, better light output over lamp life, and better color?

Typical project costs are:

4 - T8 tubes -	\$ 8.00
Electronic ballast -	\$28.00
Labor -	<u>\$10.00</u>
Total -	\$46.00

Operating Cost Comparisons T8 vs T12 Fluorescent Systems

<u>Fixture</u> 2' x 4'	<u>Lamp Type</u> T12 Straight, 4'	<u>#/Type</u> Lamp 3 40-watt	<u>Ballast</u> Type Magnetic	<u>Watts/</u> Sq. Ft. 1.5	<u>Annual</u> Oper. Cost \$2,700
2' x 4'	T8 Straight, 4'	3 32-watt	Electronic	0.8	\$1,440
2' x 2'	T12 U-tubes	2 40-watt	Magnetic	1.5	\$2,700
2' x 2'	T8 U-tubes	3 31-watt	Electronic	0.9	\$1,620

Source: DP&L

Chart 5



Energy savings compared to T-12 system:

Savings per four-lamp fixture = 80 watts saved/hr x 4000 hours/year x \$0.06/kwh
1000 watts/kw

= \$24.00/year

So, in two years, you'll have payback.

Another Consideration

The Energy Policy Act of 1992 prohibits the manufacture of some T-12 lamps and non-electronic ballasts after 1995, however, they can still be sold. However, stocks of T12 lamps are beginning to dwindle although stocks of magnetic ballasts (the inefficient type) seem plentiful.

If you are planning new construction, watch out because some contractors will still try to use the \$12.00 magnetic ballast and T12 lamps instead of the more efficient \$28.00 electronic ballasts and T8 lamps. Another caution: ***If a contractor puts T8 lamps in a magnetic ballast system, it may be a potential fire hazard.***

Exit Signs

The exit signs in your office and plant use incandescent lamps if they have not been converted to fluorescent or LED (Light Emitting Diode) lighting. Count all your old style exit signs and then multiply by the savings shown on Chart 6. In addition to saving energy, the LED's save lamp replacement labor because they last 20 years. If you install LED retrofits, remember the following:

- Use only UL labeled kits.
- Be sure the new LED provides adequate exit lighting; a 2 watt LED exit sign is equivalent to a 40-watt incandescent and should be okay.

Incandescent vs. Fluorescent Exit Lights

Annual Cost Comparison

<u>Lamp Type</u>	<u>Bulb Retail</u>	<u>Energy Cost (\$)*</u>	<u>Annual Cost (\$)</u>
40-watt T6 Incandescent	\$1**	.04kW x 8760 x \$.06 kWh = \$21	\$31
11-watt Fluorescent	\$4***	.011 kW x 8760 x \$.06 kWh = \$6	\$10

*8,760 hours/year; \$.06 per kWh

** Average life 1,000 hours; assume 10 replacements per year

*** Average life 10,000 hours; assume one replacement per year

Source: DP&L

Chart 6



- Some fire codes might require two lamps.

LED retrofit kits for your existing exit signs which are ideal for unheated areas, and they last 20-50 years.

The new exit sign retrofits use the T5 system which employs slim 5/8" diameter lamps. These compact lamps last 10-13 times longer than traditional incandescent lamps and consume 75% less energy. Models are available in twin, triple, and quad tubes for replacement of incandescents. But LED exit signs last 20 times longer and use 1/20 the energy of incandescent exit lamps.

Night Lights

Consider replacing 20- or 40-watt incandescent ceiling night lights with 3.5-watt compact fluorescents that plug directly into wall sockets - saving energy lasting 10-20 times longer than incandescent lights.

Reflectors

If your current fluorescent lamps are "bare" (no reflectors), consider installing reflectors in common strip-light fixtures. Fluorescent fixtures emit light in all directions from the lamp, including into the fixture. Optical reflectors are designed to reflect the wasted light downward.

You can also save energy by removing a portion of the lamps in each fixture. If you do delamp, remember to disconnect unused ballasts or they will continue to use energy.

When installing new fixtures, consider a three-lamp fixture with a parabolic reflector. These will deliver as much light as a four-lamp fixture with a standard reflector.

The above lighting discussions are only an introduction to office lighting energy conservation. More sophisticated approaches might include:

- Two or multiple level switching.
- Step and/or continuous dimming.
- Occupancy sensors.
- Day lighting, sky lighting, sun tubes.
- PLC lighting controllers.
- Solar shades.

PLANT LIGHTING

The yardstick for lighting efficiency is lumens/watt. The lumen is the international unit of the quantity of light. Lumens/watt express the luminous efficacy of a light source: light output divided by power input. Examples of the efficacy or effectiveness of various light source is shown on Chart 7.

HID

Because of their efficiency, High Intensity Discharge (HID) are used in most industrial plants. HID's produce more light per watt than other sources - and last longer, up to 24,000 hours. Types of HID lamps include:

- Mercury Vapor (MV) - least efficient, green or blue green light.
- Metal Halide (MH) - good efficiency, good color rendition, excellent for outdoor security lighting. Used for entry and display lighting where color rendition is important.
- High Pressure Sodium (HPS) - very efficient, warm, golden light, more suitable for outdoor but used for indoor lighting where color rendition is unimportant.

Lumens Per Watt

<u>Light Source</u>	<u>Lumens/Watt</u>
Edison's first lamp	1.4
Fluorescent lamps	45-100
Infrared lamps	6-9
Incandescent lamps	8-18
Halogen lamps	12-28
Mercury lamps	42-100
Metal Halide lamps	80-115
High pressure sodium lamp	50-140
Theoretical max. for white light	220t

Source: Plant Service

Chart 7



- Low Pressure Sodium (LPS) - high efficiency limited yellow tone light that renders colors poorly. LPS lamps are limited to outdoor and security applications.

Energy Saving Opportunities

- Replace interior incandescent and mercury vapor lamps with **metal halide lamps** where identifying colors is important.
- Replace interior incandescent and mercury vapor lamps with **high pressure sodium** where color is not important.
- Replace incandescent and mercury vapor in exterior locations with **metal halide, high pressure sodium** and/or **low pressure sodium lamps**. For good color use metal halide.
- Replace incandescents with **screw-in high pressure sodium**, for substantial energy savings and only 30 seconds restrike time.
- NOTE: When replacing mercury vapor lamps with metal halide or sodium lamps, the ballasts must be changed.

Chart 8 shows the savings for the various potential replacement schemes.

Explosion Proof Area

Remember that when specifying lighting fixtures that the following requirements apply according to NFPA 36 (1997 Edition) and the NFPA 70, National Electric Code (1996 Edition).

- Bulk Storage & Solvent Unloading – Class I, Group D, Division I or II*.
- Preparation and Meal Finishing and Receiving – Class II, Group G, Division I or II*.
- Extraction – Class I, Group D, Division I or II*.

* Depending on the location in the process.

Lamp Comparison Chart

If you are using....	Retrofit with....	Watt Savings per Lamp*	Annual \$ Saved** (10 lamps)
Mercury Vapor			
100 watt	50-watt HPS	59	\$ 129
	35 watt LPS	65	\$ 142
175 watt	100 watt HPS	70	\$ 153
	100 watt MH	70	\$ 153
	90 watt LPS	80	\$ 175
250 watt	150 watt HPS	77	\$ 169
	175 watt MH	67	\$ 147
	135 watt LPS	99	\$ 217
400 watt	250 watt HPS	140	\$ 307
	250 watt MH	150	\$ 329
	135 watt LPS	272	\$ 596
1,100 watt	400 watt HPS	610	\$1,357
Metal Halide			
100 watt	70 watt HPS	40	\$ 88
	55 watt LPS	53	\$ 116
175 watt	100 watt HPS	75	\$ 164
	90 watt LPS	85	\$ 186
250 watt	150 watt HPS	100	\$ 219
	135 watt LPS	122	\$ 267
400 watt	250 watt HPS	150	\$ 329
	150 watt LPS	240	\$ 526

Source: DP&L

Chart 8



** Using \$0.06 per kWh and 3,650 operating hours per year

* Watt savings will not necessarily equal original lamp voltage minus new lamp wattage, due to differences in ballasts

One leading oilseed processing company always specifies Division I (the most severe) for all explosion-proof locations. In that way, they eliminate possible "division" confusion and mixups.

Relamping

When you relamp your offices, yard, plant, warehouses, and other incandescent, mercury vapor, and fluorescent lamp areas - and you should! - there will be a net positive impact on profits. But, when you remove all those old T12 lamps and mercury vapor lamps, you now have to be careful about disposal. Ballasts made before the late 1970's probably contain PCB's. Fluorescent and HID lamps contain mercury.

Generally, you can identify PCB ballasts as shown on Table 3.

BALLAST IDENTIFICATION
<ul style="list-style-type: none">▪ All ballasts manufactured through 1979 may contain PCB's.▪ Ballasts manufactured after 1979 that do not contain PCB's are labeled "NO PCB's".▪ If a ballast is not labeled "No PCB's" assume it contains PCB's.

Table 3

There are numerous federal regulations that the EPA uses to control lamp and ballast disposal, including the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA); the Toxic Substances Control Act (TSCA); the Resource Conservation and Recovery Act (RCRA); and you should look at the special regulations of your state.

The good news is that you may be a **CESQG** (Conditionally Exempt Small Quantity Generator) which is a generator who generates 100 kilograms or less per month of hazardous waste, which exempts you from certain **RCRA** regulations.

In any event, it is suggested that you contact your local EPA office shown in Appendix 1.

Green Lights

You should also become familiar with **Green Lights**, an exciting and innovative program sponsored by the U.S. Environmental Protection Agency (EPA) that encourages companies to install energy-efficient lighting technologies. For information contact:

Green Lights Program
U.S. EPA
401 M Street, SW (6202J)
Washington, DC 20460

or

Green Lights Hotline:

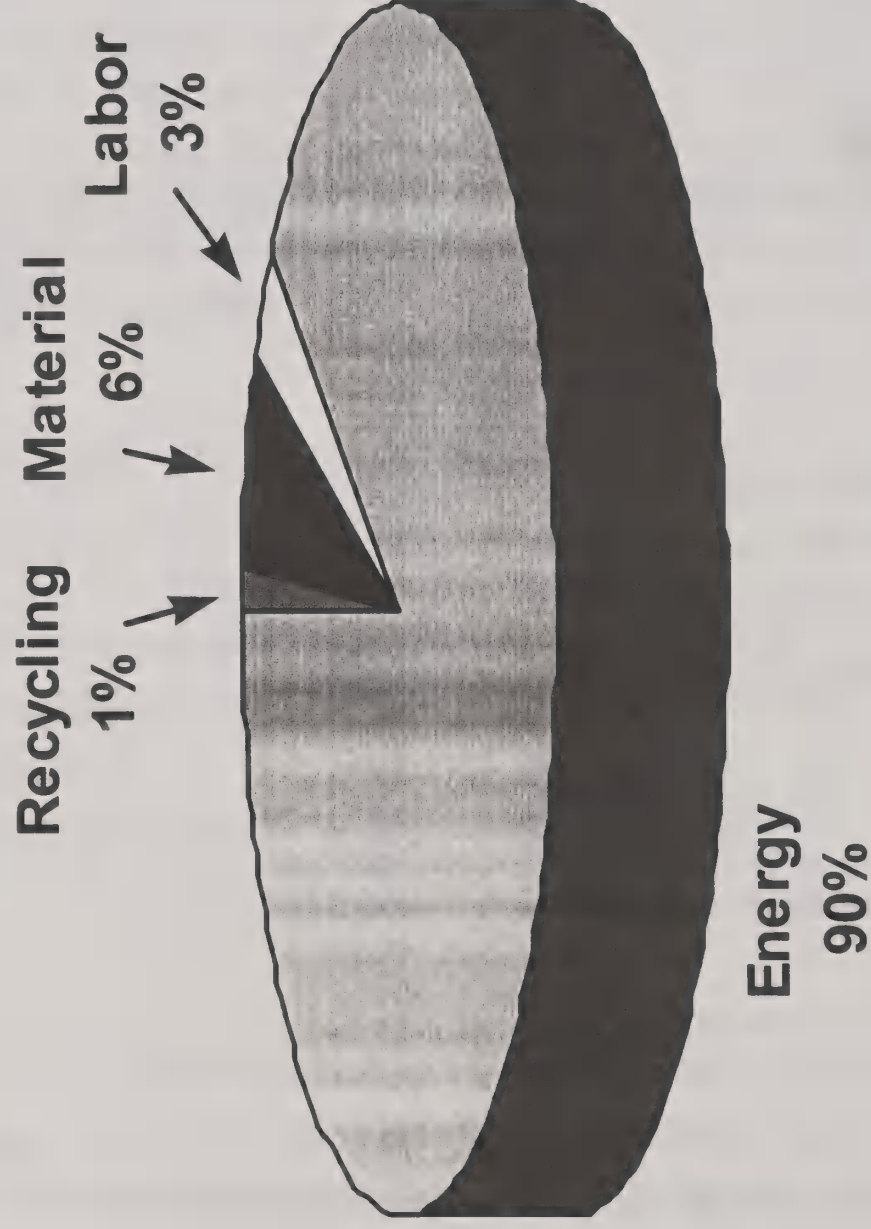
TEL: 1-888-STAR-YES
FAX: 1-202-775-6680

or

Green Lights Homepage: WWW.epa.gov/greenlights.html

The proper disposal of old lamps has little impact on the financial return of upgrading your lighting under Green Lights recommendation. Chart 9 shows that the impact of lamp disposal on the profitability of typical Green Lights lighting

Fluorescent Lamp Life-Cycle Cost



Source: EPA

Chart 9

upgrade projects is minimal. The example below shows the impact of various lamp recycling costs on the internal rate of return (IRR) and the net present value (NPV) of a typical lighting upgrade project. The assumed project consists of upgrading a four-lamp standard fluorescent system that uses magnetic ballasts and 40-watt lamps with a four-lamp T8/electronic system and occupancy sensors. Without considering the cost of lamp disposal, the IRR and NPV were calculated at 47.1% and \$52,242, respectively. Note that even when assuming lamp disposal costs of \$1.50 per lamp - three times the average recycling cost - the IRR and NPV values decreased only slightly to 44.8% and \$51,642 respectively. These results were obtained using the Green Lights analysis tool *ProjectKalc*.

Fluorescent Lamp Life-Cycle Cost

There are three considerations concerning the cost of old lamp disposal:

1. Operating a lamp for the normal 20,000 hours at \$.07/kwh is \$64.00. Therefore, a \$.50 disposal cost is insignificant.
2. Replacing an old four-lamp T12 fixture with a T8 will cost \$50 to \$60 including installation, plus \$2.00 disposal (per 4 lamps). If the new fixture uses 50% of the electricity of the old fixture (which is typical), the savings will pay for the cost of disposing of the old lamps after about 310 hours - about one month for most businesses! The total new T8 installation should save about \$24/year and you will have a payback period of 2.0 to 2.5 years.
3. Environmentally, T8 lamps are better. See Chart 10 for the difference in mercury levels. Your changing to T8 lamps will help the environment.

Mercury Levels

- T8 lamps contain about 15 mg of mercury compared to 20-30 mg for T12 lamps
 - so less mercury is disposed of during relamping
- T8 lamps are more energy efficient than T12 lamps
 - so less mercury is emitted from fossil-fueled generating plants

Source: EPA

Chart 10



PLANT OPERATIONS

The largest potential energy savings in an oilseed crushing plant are in the preparation and solvent extraction operations. In the previous discussion of T.O.P., it was stressed that there are many places to look for savings in the "T" - transportation and "O" office. Management should show commitment in its own "backyard," particularly in the office, and promote Energy Management in HVAC and lighting. If management demonstrates a distinct enthusiastic commitment to energy conservation, employees in all sections of the organization will support energy conservation, and will think of new ways to save in their individual areas of responsibility.

When your Energy Management program begins in the office, then the plant personnel will be more receptive to becoming energy savers.

The processing plant is where the big energy cost dollars reside. The two primary costs are electricity and steam.

Steam is the largest energy cost, particularly for a solvent extraction plant. It is a large enough subject, however, to be discussed separately. Therefore, this paper will be limited to electrical energy. The paper entitled Environmental Impact of Energy Losses in Oilseed Crushing Operations, noted in the REFERENCES, discusses various ways of saving **steam** energy in the extraction plant.

Process Electricity

Process electrical power, primarily for motors, is controllable and manageable. However, unlike the lighting and electrical heating load in your office and plant, process electricity is three phase power and additional costs must be understood - primarily power factor and demand changes.

Power Factor

Electric motors, vibrating feeders, transformers, and solenoids all have wound magnetic coils. Two different components of power flow through these coils:

1. Kilowatts (kw) - this is the energy for **work done** and is measured by the watt meter.
2. Reactive Kilovolt - amperes KVAR - this is the reactive current required to generate a magnetic field around the coils of motors, solenoids, etc. The KVAR component does **no useful work** and **is not measured by the watt meter**. However, the KVAR component of power is an energy loss since it heats generators, transformers, and electrical transmission lines. You can be sure that your electric utility is very interested in Power Factor.

Power factor is expressed as PF:

$$\text{PF} = \frac{\text{Useful power}}{\text{Total power}} = \frac{\text{KW}}{\text{KVA}}$$

A light bulb, electric heater, or other non-inductive device has a power factor of 1.0. A motor, which is an inductive device, will typically have a power factor 0.3 to 0.9, depending on its design and at what percent of full load it is operating. This is shown on Chart 11.

If you have a 100 HP with a full load ampere (FLA) rating of 124 FLA and it is operating at 30 amperes, the motor is at 25% of full load. From Chart 12, the apparent power factor would be 50%. If you have enough of these situations operating long enough, it will seriously affect the Demand Billing portion of your power bill.

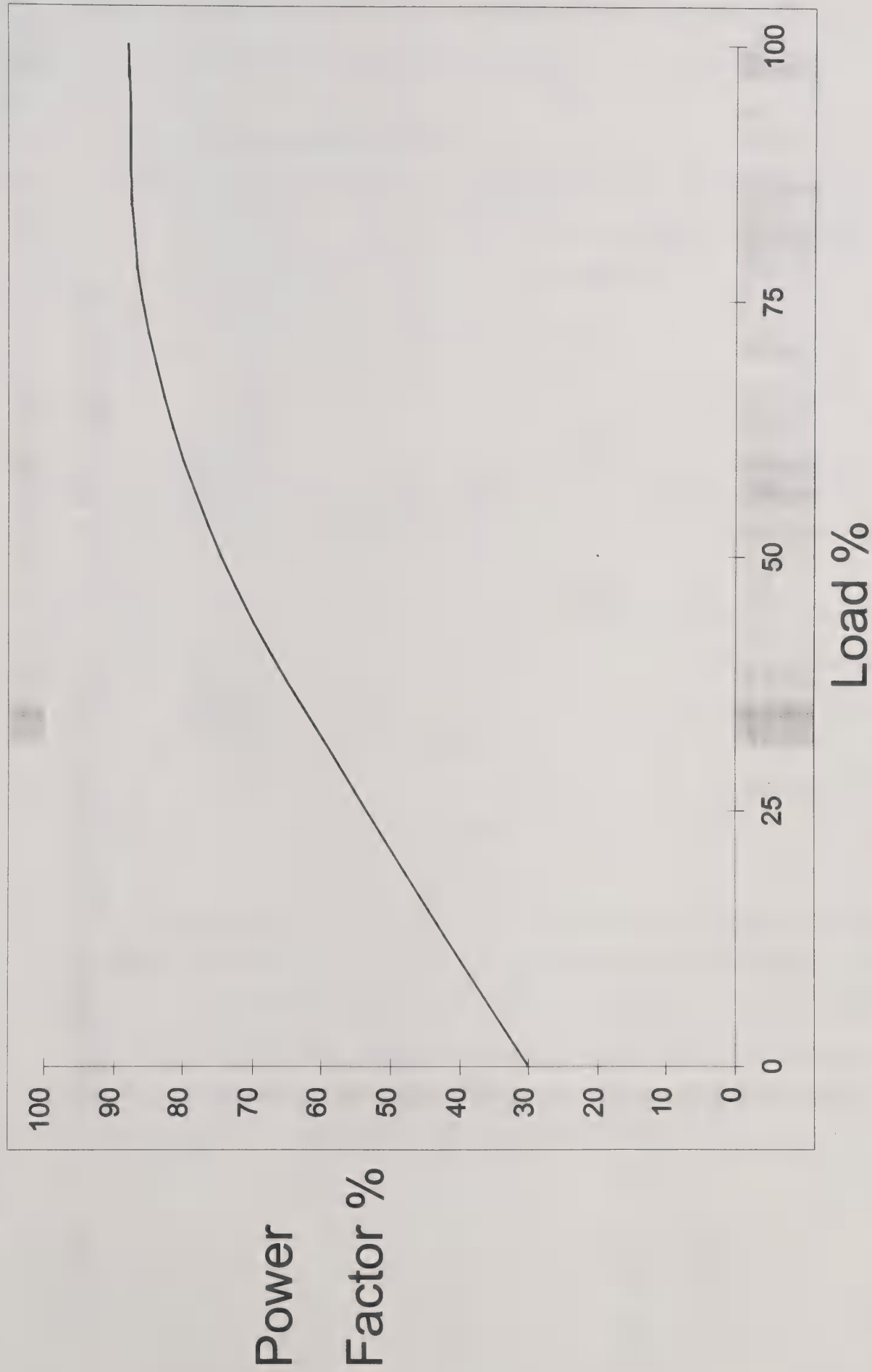


Chart 11 Power Factor of Industrial Motors

Source: AFMA

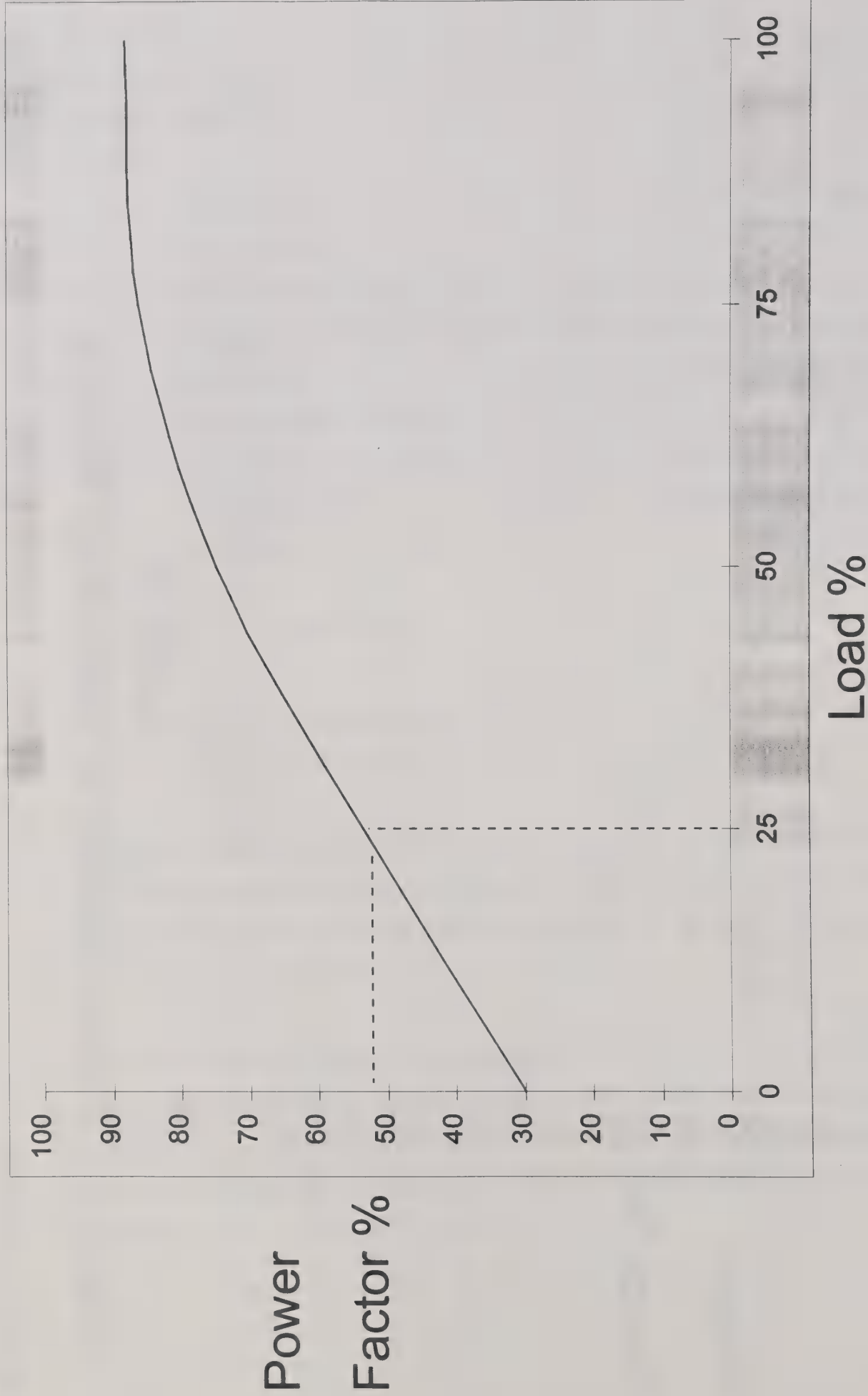


Chart 12 Power Factor of Industrial Motors

Source: AFMA



It is important that you understand how your utility is billing you. A typical primary billing from the Dayton Power and Light Company would include the following charges/month for three-phase service at the primary rate:

1. Customer charge - \$95.00
2. Demand charge for all kw of billing demand - \$13.8623/kw
3. Demand charge for all KVAR of billing demand - \$.30/KVAR
4. Energy charge for kwh used - \$.00824/kwh
5. Fuel cost - \$.01201/kwh
6. PIP cost - \$.00031/kwh

These rates are based on primary voltage service. Primary voltage customers own the transformers and have responsibility for transformer maintenance.

The demand charge is based on the greatest (30) minute integrated kw demand during the month. The billing demand is either 75% of the off-peak demand or 100% of demand for peak periods - 8:00 a.m. to 8:00 p.m., Monday through Friday, not including six legal holidays.

If the kilovars are not measured, a 90% power factor is assumed.

This is just the way one utility, DP & L, handles billing, and each utility will vary its rates. The lesson to be learned is that you must understand your electric bill. Customer-oriented utilities like Dayton Power and Light are always pleased to discuss how you are billed, along with ways to reduce your energy usage. It is actually better business for electric utilities to help their customers reduce energy than to be forced to build new power-generating plants!

Electric Motors

Once the billing system of your utility is well understood, then you can begin managing the efficient use of motors.

The electric motor-driven equipment - Conveyors, cracking mills, flaking mills, conditioners, DTDC's, air compressors, hammermills, pumps, and fans - are the most power inefficient and, at the same time, the largest users of electrical power in your plant. This is where the potential savings are.

Incoming Power

Monitor your incoming power to be sure that you have the **proper voltage**, along with **balance between phases**. **Reduced voltage** causes the motor to draw more current which increases the stator and rotor losses.

The typical problem is **over voltage**. Motors operating at higher than 5-10% of nameplate voltage have reduced efficiency and reduced life due to insulation breakdown or phase imbalance.

Imbalances in voltage between phases will increase motor losses and decrease insulation life. The usual causes are faulty power factor correction equipment, inadequate transformer capacity, single-phase-to-ground faults, open circuits in the distribution system primary, and, possibly, problems that are utility generated.

Another cause of phase imbalance could be unevenly distributed single-phase loads on the same power system. Imbalances of 2-3% can cause loss of equipment life.

Transformers

Depending on the rate structure of your own utility, transformer ownership should be considered. Once a processing plant consumes more than one megawatt/month, ownership of a transformer and becoming a primary rate customer could save energy dollars and should be investigated.

Capacitors

If you are considering increasing your electrical load and your transformer is nearing its peak capacity, the installation of capacitors in your plant could help correct your power factor and lower your KVA demand. This could increase the capacity of your transformer which is sized for KVA demand.

Energy Efficient Motors

The decision of whether or not to use energy-efficient motors has been made for you!

EPACT

The U.S. Energy Policy Act of 1992 or EPACT has mandated that energy efficient motors are now required in the United States in the future for new motors placed into service. The law goes into effect as follows:

- Standard Motors = October 24, 1997
- Explosion Proof Motors = October 24, 1999

The requirement extends to not only motors manufactured in the U.S., but also to motors exported to the U.S.

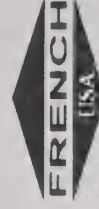
The new EPACT-required Efficiency Levels are shown on Chart 13. Note that explosion-proof motors are not covered until October 24, 1999. In the interim, explosion-proof motors manufactured with the old efficiency ratings are allowed. Your motor distributor might offer you an old standard explosion proof motor

Electric Motor Efficiency Levels Prescribed in the Energy Policy Act of 1992

Motor HP	6 Poles	4 Poles	2 Poles
1	80.0	82.5	75.5
1.5	85.5	84.0	82.5
2	86.5	84.0	84.0
3	87.5	87.5	85.5
5	87.5	87.5	87.5
7.5	89.5	89.5	88.5
10	89.5	89.5	89.5
15	90.2	91.0	90.2
20	90.2	91.0	90.2
25	91.7	92.4	91.0
30	91.7	92.4	91.0
40	93.0	93.0	91.7
50	93.0	93.0	92.4
60	93.6	93.6	93.0
75	93.6	94.1	93.0
100	94.1	94.5	93.6
125	94.1	94.5	94.5
150	95.0	95.0	94.5
200	95.0	95.0	95.0

Source: EPACT

Chart 13



either from the manufacturer or from stock at a good deal: Is it a good deal for you?

If you need to replace an old 50 HP motor or purchase a new piece of equipment with a 50 HP motor, what should you do? Specify “old” efficiency, EPACT nominal efficiency or premium efficiency?

The following example shows you how to calculate the payback:

Assume: 50 HP Explosion Proof, 1200 RPM running 24 hours/day, 7 days/week, 50 weeks/year at 100% load. Also assume a \$520 difference in motor cost (\$3560-\$3040). “Old” standards efficiency is 91.7%. A Premium Efficient motor is 94.7% efficient.

$$\begin{aligned}\text{KW Saved/HR:} &= \text{HP} \times 0.746 [1/\text{std. Eff.} - 1/\text{prem/eff.}] \times \text{Load} \\ &= 50 \times 0.746 [1/.91 - 1/.95] \times 100\% \\ &= 37.3 [1.0905 - 1.0560] \times 1.00 \\ &= 1.287 \text{ KWH}\end{aligned}$$

$$\begin{aligned}\$ \text{ Saved/YR:} &= 1.287 \text{ KWH} \times 8400 \text{ hrs} \times \$0.06 \text{ KWH} \\ &= \$648.65\end{aligned}$$

$$\begin{aligned}\text{PAYBACK:} &= \text{Cost difference/Savings} \times 12 \text{ months} \\ &= \$520/\$648.65 \times 12 \text{ months} \\ &= 9.6 \text{ month payback}\end{aligned}$$

The above payback analysis is for a 50 HP motor. But you can substitute the specifications and efficiencies of any motor in a similar analysis.

The payback analysis on replacing an existing motor would, of course, show a longer payback period and might not be economically feasible. When analyzing the payback for replacing an existing motor, consider the following:

- Cost of removing the existing motor.
- Down time.
- Cost of installing the new motor.
- Salvage value of the old motor.
- Time value of money since the payoff period will be longer.
- Tax advantages or disadvantages including potential energy tax credits and depreciation recapture.

Flaking

The flaking operation is the largest consumer of electricity in the crushing operation, offering energy-saving potential. In the 1200 MTD soybean plant, flakers represent 32% of the total connected HP. The principle variable affecting flaker HP is flake thickness.

FLAKER HORSEPOWER IS INVERSELY PROPORTIONAL TO FLAKE THICKNESS.

Flake thickness for soybean processing varies from 0.010 to 0.016 inches (0.25 to 0.41 mm). When planning a new plant or the expansion of an existing one, consideration of producing thicker flakes can result in significant energy savings. Consider the cost savings including roll maintenance and power when producing 0.11 inch (0.28 mm) flakes vs. 0.15 (0.38 mm) flakes for the 1200 MTD plant:

Cost for 0.11" Flakes: \$0.54/ton

Cost for 0.15" Flakes: \$0.40/ton

DIFFERENCE: \$0.14/ton

SAVINGS/YR = \$0.14 x 1200 MTD x 350 day/hr.

= \$58,800 / year

Fans and Pumps

After the flaking energy, the next largest consumers of electricity are centrifugal pumps and fans. These devices operate according to the “Affinity” Laws or “Fan Laws,” which are:

- Flow (cfm or gpm) is proportional to the speed (rpm).
- Pressure (inches Hg or psi) varies as the square of the speed (rpm).
- Power (HP) varies as the cube of the speed (rpm).

Adjusting flow by means of speed control instead of dampers or valves can save energy. Chart 14 shows power vs. flow relationship. For example, if you reduce flow 20% by reducing rpm 20%, you reduce power by over 48%.

Therefore, many users of centrifugal pumps and fans that have reduced flow requirements for extended times can save energy by installing variable speed systems such as A/C variable frequency drives on these devices.

A word of caution: Compatibility of the inverter with the motor, especially an energy-efficient motor, is critical. It is best to purchase a packaged drive system rather than “mix and match” manufacturers. Additionally, be sure that variable frequency motors in an extraction plant maintain their TSB temperature code at minimum speed.

When designing a new fan system, it is important to match the fan with the system so that efficiency peaks at the desired system point. Incidentally, a fan produces the least amount of sound when it operates at its point of peak efficiency.

Fan Flow vs. Energy Consumption

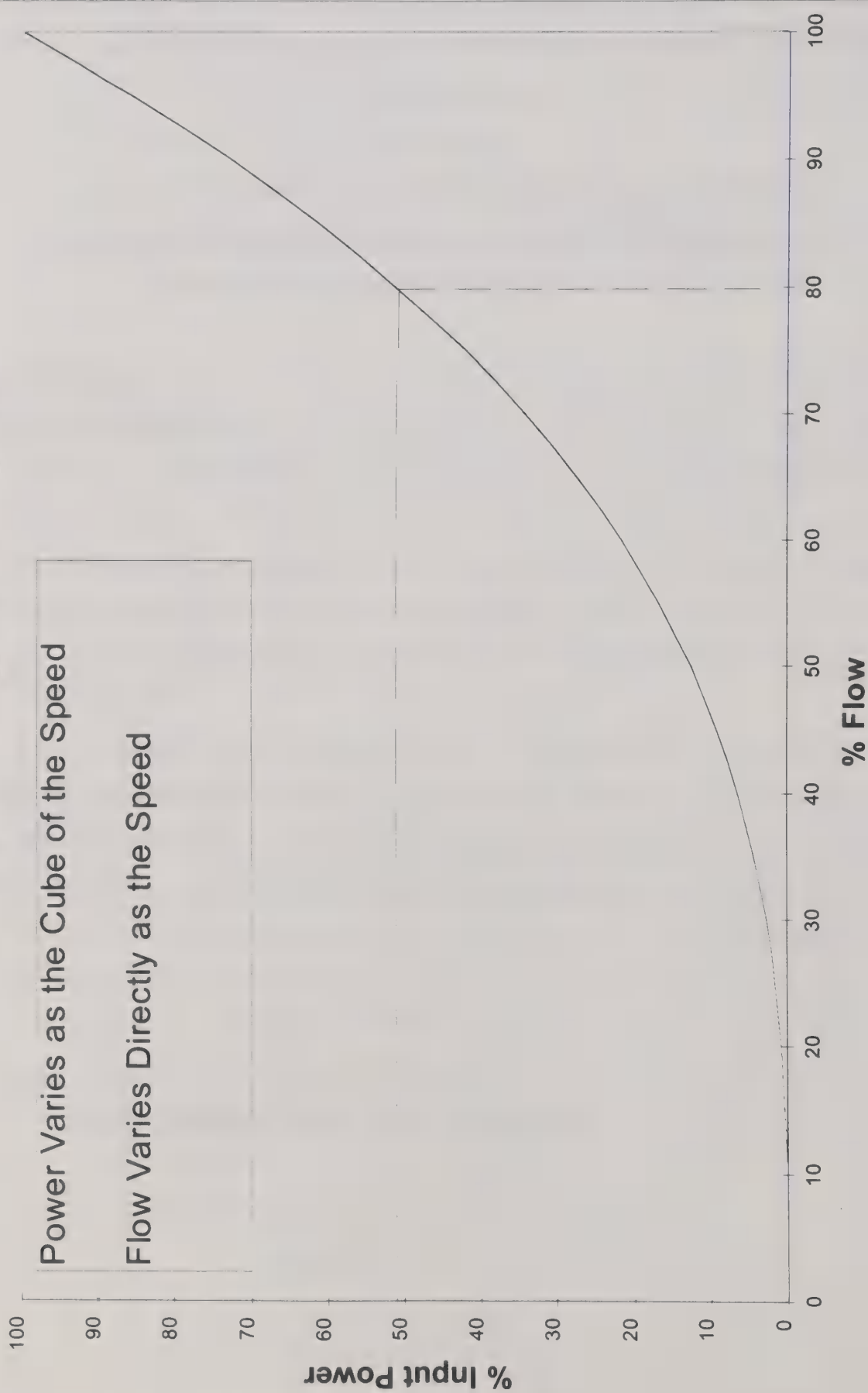


Chart 14



In addition to specifying the correct fan, it is very important to use the correct type of fan blade. For example:

A XYZ fan with a 50-1/2" radial blade with rim producing 24,000 cfm @ 30" Static pressure requires 177 BHP

Whereas:

A Buffalo Forge fan with a 44.5" backwardly inclined blade producing 24,000 cfm @ 30" static pressure requires 137 BHP

A savings of 40 HP or 22% - for same air output.

The radial blade is less expensive, easy to manufacture, and is an excellent choice where the fan sees a heavy material loading.

The backwardly inclined blade is quieter, more expensive, and more efficient, and is a good choice for light dust loading.

So, for example, if you are blowing dust into a cyclone, put an airlock on the cyclone and use a fan with a backwardly inclined blade on the exhaust side of the cyclone.

Air Compressors

The Compressed Air System in a plant is an energy user but is frequently overlooked when investigating sources of avoidable energy loss.

Air Leaks

All compressed air systems leak and continued inspection and maintenance is necessary. If you hear a hiss - it's a leak! Chart 15 shows the kwh wasted from various sized holes.

Operating Pressure

Make a sketch or drawing of your entire compressed air system. It has undoubtedly changed since originally installed. Record the pressure required at each use point. Strategically located pressure gages can monitor use points and alert you if a pressure drop occurs which requires system maintenance.

Otherwise, complaints of inadequate pressure occur, and the "quick fix" is to increase overall system pressure. However, energy costs also increase!

The goal is to lower discharge pressure, if possible, without affecting the efficient operation of equipment. Chart 16 shows the relationship between lowering air pressure and the decrease of horsepower.

Some plants have specific operations that require higher pressure than the remainder of the facility. The possibility of using a point-of-use compressor or booster system should be investigated. Then the main system pressure can be reduced and energy saved.

Compressor Control

If your plant has independently controlled multiple compressors, you are wasting energy! A multiple compressor control system can reduce energy by up to 10%.

Inlet Air

The source of intake air is very important. Cooler air is denser and already partially compressed. The intake air should be filtered in the coolest spot available, preferably outside the building. A north-facing location is often the best. The worst location would be near a paved asphalt parking lot in a sunny

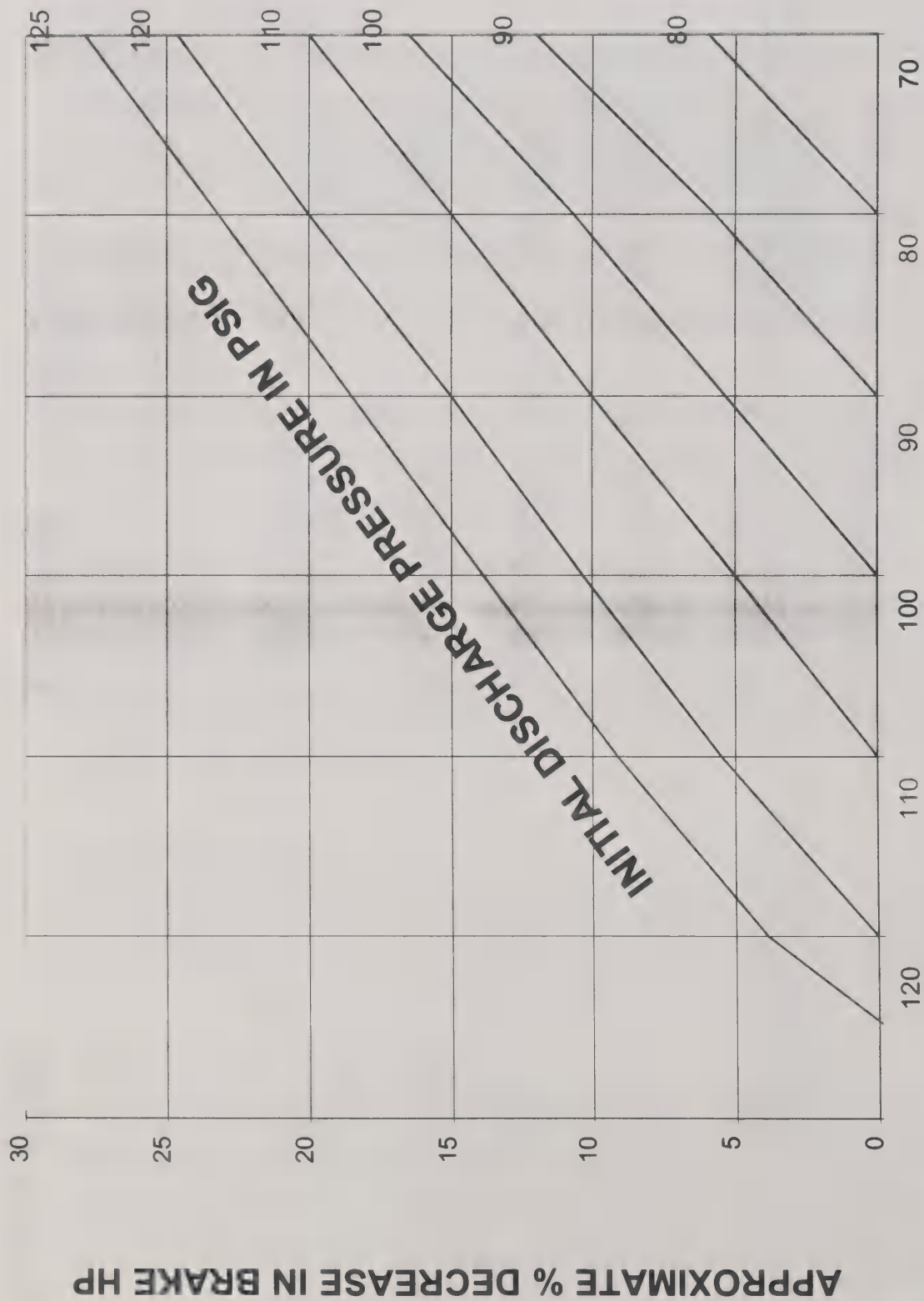
Effect of Air Leaks

<u>Hole Dia. Inches</u>	Annual Cu.Ft. Free Air <u>79,900,000</u>	<u>Kilowatt Hours</u> 219,000
3/8		
1/4	35,500,000	97,200
1/8	8,880,000	24,000
1/16	2,220,000	6,060
1/32	553,000	1,510

Source: AFMA

Chart 15





LOWERED DISCHARGE PRESSURE PSIG



Chart 16

Source: Dow Chemical

location. Extending your compressor intake air to the outside can be very cost effective.

Heat Recovery

The electrical energy for air compression is consumed as follows:

Energy into the compressed air - 20%

Energy converted to heat - 80%

This represents an opportunity to recover about 50,000 btu/hr per 100 cfm of intake air for air-cooled compressors.

Heat recovery for water-cooled compressors is more complex and costly, but should be analyzed for compressors over 125 HP.

Air Dryers

The first step in producing dry air is the after-cooler. Use an oversized air cooler since this is a low-energy method of water removal. However, even though moisture is removed from the air, the temperature is lowered resulting in cooled air at 100% relative humidity. Further drying is usually required.

The types of dryers generally used are refrigerant dryers, desiccant dryers, and re-heaters.

The features are:

Refrigerant Dryers - Dries air to a 35° dew point. Relatively energy efficient. Gives best results at 50° F to 100° F ambient.

Desiccant Dryers - Dries air to -40° F dew point. Very energy intensive. Required where compressed air systems are exposed to temperatures below 32° F.

Re-Heaters - Raises the air temperature thus lowering the relative humidity of the compressed air with "free" heat from the compressor. Only suitable for systems capable of using warmer air.

The end use of the air combination of air dryers should be considered. If cooled water is available for the after-cooler (as it is in a crushing operation), and the specific system is within a heated building, the air from the after-cooler may be sufficiently dry for many applications.

Metering

The execution and result measurement of energy savings projects can be greatly helped by installing secondary watt meters in the various cost centers. If the purchase of new meters cannot be justified, most utilities will lend meters or provide them at a "friendly" rental price. In this way, you can accurately measure power usage before and after.

Monitoring

An important part of a good Energy Management Program is the ability to monitor excessive demand, severe voltage fluctuations, unbalanced loads, etc., with a computer-based monitoring system. Some utilities can offer you a monitoring service. DP & L, for example, offers the Entelligence System for a modest fee for each monitoring point. To quote Ken Bailey, Senior Engineer with the Dayton Power & Light Company: "You can only manage what you can monitor."

CONCLUSION

Today, I have given you just a few ways to manage energy.

***It is the right thing to do for your business,
it is the right thing to do for your children and grandchildren,
it is the right thing to do for the world.***

As my old Latin professor was fond of saying: ***Pila in area tua est****

* The ball is in your court!

ACKNOWLEDGEMENTS

The author extends special thanks to the Energy Resource Center of the Dayton Power and Light Company for providing research material for this paper, especially Gayle Sampson, Research Specialist, and Ken Bailey, Registered Architect and CEM (Certified Energy Manager).

REFERENCES

Ambler, Robert F., Compressed Air Systems, Feed Manufacturing Technology IV, pp. 320-21

Bailey, Kenneth, Lighting Control for Commercial Facilities, proceedings Lighting Seminar, DP & L Energy Resource Center, July 14, 1997

Dunavent, Richard L., Engineered Air Products, private communications with R.L. Stroup, January 1998

Farmer, Richard, Vice President Operations, Bunge USA, private communications with R.L. Stroup, January 1998

Footlik, P.E., Robert B., Save Energy in Material Handling, Plant Services, January 1997, pp 55-56

Kemper, Timothy G., Deep Bed Solvent Extraction, Texas A & M University Vegetable Oil Short Course, May 1996

NFPA 36, Standard for Solvent Extraction Plants, 1997 Edition

Reliance Electric, What You Should Know About the U.S. Energy Policy Act and Electric Motors, 1993

Revelt, Jean J., The Ins and Outs of Motor-Driven Systems, Plant Services, November 1996, pp 50-61

Reynolds, Bruce J., USDA, Rural Business-Cooperative Service, Cooperative Cottonseed Oil Mills, May 1997

Rorer, Paul, It Used To Be So Simple!, Plant Services, December 1997, pp 18

Saxton, Paul, Optimum Fan Performance: What It Is and What It Is Not, Plant Services, February 1997, pp 23-25

Stroup, Robert L., Environmental Impact of Energy Losses in Oilseed Crushing Operations, proceedings World Conference on Environmental Challenges, March 4-7, 1997, Brussels, Belgium

Toshiba International, The Impacts of The Energy Policy Act of 1992 on Industrial End Users of Electric Motor-Driven Systems

USEPA, Lighting Waste Disposal, EPA 430-B-95-004, February 1997

Welch, David, Reducing Energy and Costs, Plant Services, July 1996, pp 63-68

APPENDIX I

INFORMATION RESOURCES

EPA Regional Offices

REGION I (ME, VT, NH, MA, CT, RI)

Environmental Protection Agency
John F. Kennedy Federal Building
Room 2203
Boston, MA 02203
(617) 565-3420

REGION II (NY, NJ, PUERTO RICO, VIRGIN ISLANDS)

Environmental Protection Agency
Jacob K. Javits Federal Building
26 Federal Plaza
New York, NY 10278
(212) 264-2657

REGION III (PA, WV, VA, MD, DE, WASHINGTON DC)

Environmental Protection Agency
841 Chestnut Building
Philadelphia, PA 19107
(215) 597-9800

REGION IV (TN, KY, NC, SC, GA, AL, MS, FL)

Environmental Protection Agency
345 Courtland Street, NE
Atlanta, GA 30365
(404) 347-4727

REGION V (IL, WI, IN, MI, MN, OH)

Environmental Protection Agency
77 West Jackson Boulevard
Chicago, IL 60604-3507
(312) 353-2000

REGION VI (NM, TX, OK, AR, LA)

Environmental Protection Agency
First Interstate Bank Tower at Fountain Place
12th Floor/Suite 1200
1445 Ross Avenue
Dallas, TX 75202-2733
(214) 665-6444

REGION VII (NE, KS, MO, IA)

Environmental Protection Agency
726 Minnesota Avenue
Kansas City, KS 66101
(913) 551-7000

REGION VIII (MT, WY, ND, SD, UT, CO)

Environmental Protection Agency
Suite 500
999 18th Street
Denver, CO 80202-2405
(303) 293-1603

REGION IX (CA, NV, AZ, HI, AMERICAN SAMOA, GUAM)

Environmental Protection Agency
75 Hawthorne Street
San Francisco, CA 94105
(415) 744-1305

REGION X (WA, OR, ID, AK)

Environmental Protection Agency
1200 Sixth Avenue
Seattle, WA 98101
(206) 553-4973

State Solid and Hazardous Waste Agencies

ALABAMA

Department of Environmental Management
Land Division — Solid/Hazardous Waste
1751 Federal Drive
Montgomery, AL 36130
(205) 271-7761/7735

ALASKA

Steve Willingham
Manager, Solid Waste Program
State of Alaska
Department of Environmental Conservation
410 Willoughby Avenue
Juneau, Alaska 99801-1795
(907) 465-5158

ARIZONA

Anthony Leverock
Arizona Department of Environmental Quality
Hazardous Waste Permits Unit
3033 North Central Avenue
Phoenix, AZ 85012
(602) 207-4160

ARKANSAS

Bob Finn
Department of Pollution Control and Ecology
Hazardous Waste Division
PO Box 8913
Little Rock, AR 72219-8913
(501) 562-7444
Fax (501) 562-6532

CALIFORNIA

Mardis Coers
Department of Toxic Substances Control
PO Box 806
Sacramento, CA 95812-0806
(916) 322-0712

COLORADO

Scott Klarich
Environmental Compliance Officer
Monitoring and Enforcement Section
Hazardous Materials and Waste Management Division
Colorado Department of Health and Environment
Mail Code: HMWMD-HWC-B2
4300 Cherry Creek Drive South
Denver, CO 80222-1530
(303) 692-3369

CONNECTICUT

Department of Environmental Protection
Waste Management Bureau
79 Elm Street
Hartford, CT 06106
(203) 566-8476

DELAWARE

Department of Natural Resources and Environmental
Control
Division of Environmental Control
Solid Waste/Hazardous Waste Section
Edward Tatnall Building
PO Box 1401
Dover, DE 19901
(302) 739-4403

Delaware Solid Waste Authority
PO Box 71
New Castle, DE 19901
(302) 736-5361

DISTRICT OF COLUMBIA

Department of Consumer and Regulatory Affairs
Environmental Regulation Administration
Pesticides, Hazardous Waste and Underground
Storage Tank Division
Hazardous Waste Management Branch
(Hazardous Waste Disposal)
2100 Martin Luther King, Jr. Ave. SE,
Suite 203
Washington, DC 20020
(202) 404-1167

Department of Public Works
Public Space Maintenance Administration
Bureau of Sanitation Services
(Solid Waste Disposal/Recycling)
2750 South Capitol St., SE
(202) 767-8512

FLORIDA

John Price
Bureau of Solid and Hazardous Waste
Department of Environmental Protection
2600 Blair Stone Road
Tallahassee, Florida 32399-2400
(904) 488-0300

GEORGIA

Vern George
Environmental Protection Agency
Toxics Branch
345 Courtland St., NW
Atlanta, GA 30334
(404) 347-1033

Department of Natural Resources
Environmental Protection Division
Land Protection Branch
205 Butler Street, SE
Suite 1154
Atlanta, GA 30334
(404) 656-2833

HAWAII

State of Hawaii
Department of Health
Environmental Management Division
Clean Air Branch
Asbestos Abatement Office
PO Box 3378
Honolulu, HI 96801-3378
(808) 586-8144

IDAHO

William Fritell
Department of Health and Welfare
Division of Environment
Bureau of Hazardous Materials
450 W. State Street
Boise, ID 83720
(208) 334-5879

ILLINOIS

Edwin Bakowski
State of Illinois
Environmental Protection Agency
2200 Churchill Road
Springfield, IL 62794-9276
(217) 524-3300

INDIANA

Robert Snodgrass
Solid Waste Permit Section
105 South Meridian Street
Indianapolis, IN 46206-6015
(317) 232-5976

IOWA

Lavoy Haage
Department of Natural Resources
Solid Waste Section
Land Quality Bureau
Wallace State Office Building
900 East Grand Avenue
Des Moines, IA 50319
(515) 281-4968

KANSAS

Ron Smith
Department of Health and Environment
Solid Waste Management Division
Forbes AFB Bldg. No. 740
Topeka, KS 66620
(913) 296-1500

KENTUCKY

Abby Myer
Department for Environmental Protection
Division of Waste Management
Ft. Boone Plaza
14 Reilly Road
Frankfort, KY 40601
(502) 564-6716 x242

LOUISIANA

Department of Environmental Quality
Office of Solid and Hazardous Waste
Solid Waste Division
PO Box 44307
Baton Rouge, LA 70804
(504) 765-0355

MAINE

Department of Environmental Protection
Bureau of Oil & Hazardous Materials Control
State House Station 17
August, ME 04333
(207) 287-2651

Waste Management Agency
State House Station 154
August, ME 04333
(207) 287-5300

MARYLAND

Ed Hammerburg
Department of Environment
Toxic Operations Program
2500 Boening Highway
Baltimore, MD 21224
(410) 631-3345

MASSACHUSETTS

Victoria Phillips, Environmental Analyst
Office of Hazardous Waste
Enforcement Division
1 Winter Street
Boston, MA 02108
(617) 292-5812

MICHIGAN

Department of Natural Resources
Hazardous Waste Division
PO Box 30241
Lansing, MI 48909
(517) 373-2730

MINNESOTA

Nancy Ellefson
Minnesota Pollution Control Agency
Solid or Hazardous Waste Division
520 Lafayette Road North
St. Paul, MN 55155
(612) 296-6300

MISSISSIPPI

David Lee
Department of Environmental Quality
Office of Pollution Control
PO Box 10358
Jackson, MS 39209
(601) 961-5171

MISSOURI

Department of Natural Resources
Division of Environmental Quality
Waste Management Program
Jefferson State Office Building
205 Jefferson Street
PO Box 176
Missouri Boulevard
Jefferson City, MO 65102
(314) 751-3176

MONTANA

Don Vidrine
Department of Health and Environmental Sciences
Environmental Sciences Division
Solid and Hazardous Waste Bureau
PO Box 200901
Helena, MT 59620-0901
(406) 444-1430

NEBRASKA

Department of Environmental Control
PO Box 94877
State Office Building
Lincoln, NE 68509
(402) 471-2186

NEVADA

Colleen Crips
Bureau of Hazardous Waste
333 West Nye Lane
Carson City, NV 89710
(702) 687-5872

NEW HAMPSHIRE

Robert C. White, Chief
PCB Section
Department of Environmental Services
Air Resources Division/Toxics Management Bureau
64 N. Main St., Caller Box 2033
Concord, NH 03302-2033
(603) 271-1370

Department of Environmental Services
Waste Management Division/Compliance Bureau
6 Hazen Drive
Concord, NH 03301
(603) 271-2942

NEW JERSEY

Sandor Juhasz
NJ Department of Environmental
Protection and Energy
Hazardous Waste Regulation Program
401 East State Street
CN 421
Trenton, NJ 08625
(609) 292-8341
NJ Department of Environmental Protection and Energy
Solid Waste Management Division
840 Bear Tavern Road
CN 44
Trenton, NJ 08625
(609) 292-8341

NEW MEXICO

New Mexico Environmental Department
Harold Runnels Building
PO Box 26110
Santa Fe, New Mexico 87502

Hazardous and Radioactive Materials Bureau
(505) 827-4308

Solid Waste Bureau
(505) 827-2775

NEW YORK

Lawrence Nadler
Environmental Specialist
Division of Solid & Hazardous Materials
Bureau of Hazardous Waste Management
Technical Determination Section
New York State Department of
Environmental Conservation
Room 452
50 Wolf Road
Albany, NY 12233-7251
(518) 485-8988

NORTH CAROLINA

Department of Environment, Health, and Natural
Resources
Solid Waste Management/Hazardous Waste Division
PO Box 27687
Raleigh, NC 27611
(919) 733-2178

NORTH DAKOTA

Curtis Enckson
Division of Waste Management
1200 Missouri Avenue
PO Box 5520
Bismarck, ND 58502-5520
(701) 328-5166

OHIO

Environmental Protection Agency
Office of Solid and Hazardous Waste
PO Box 1049
1800 Watermark Drive
Columbus, OH 43266-0149
(614) 644-2917

OKLAHOMA

Ellen Bussert
Oklahoma Department of Environmental Quality
Public Information and Education
1000 Northeast 10th Street
Oklahoma City, OK 73117-1212
(405) 271-7353

OREGON

Rick Vopel
Department of Environmental Quality
Waste Management Clean-up Division
811 S.W. 6th Avenue
Portland, OR 97204
(503) 229-5630

PENNSYLVANIA

Department of Environmental Resources
Bureau of Waste Management
PO Box 8471
Harrisburg, PA 17105-8471

PUERTO RICO

Environmental Quality Board
Solid and Hazardous Waste Bureau
PO Box 11488
Santurce, PR 00910
(809) 725-5140

RHODE ISLAND

Robert Nero
Department of Environmental Management
Air and Hazardous Materials
291 Promenade Street
Providence, RI 02908
(401) 277-2797

SOUTH CAROLINA

Board of Health and Environmental Control
Bureau of Solid and Hazardous Waste
2600 Bull Street
Columbia, SC 29201
(803) 896-4174

SOUTH DAKOTA

Department of Water and Natural Resources
Environmental Health Division
Joe Foss Building
Pierre, SD 57501
(605) 773-3153

TENNESSEE

Wayne Gregory, Technical Coordinator
Department of Environment and Conservation
Division of Solid Waste Management
5th Floor, L&C Tower
401 Church Street
Nashville, TN 37243-1535
(615) 532-0780

TEXAS

Sonia Ralls
Texas Water Commission
PO Box 13087
1700 North Congress Avenue
Austin, TX 78711-3087
(512) 463-7830

UTAH

Dennis Downs
Department of Environmental Quality
Division of Solid and Hazardous Waste
PO Box 144880
Salt Lake City, Utah 84114-4880

VERMONT

Lynn Metcalf,
Department of Environmental Conservation
Hazardous Materials Management Division
103 South Main Street
Waterbury, Vermont 05671-0404
(802) 241-3888

VIRGINIA

Robert Lincoln, Waste Division
Virginia Department of Environmental Quality
Special Solid Waste Program
P.O. Box 10009
Richmond, VA 22240
(804) 527-5357

WASHINGTON

Stacie Singleton
Department of Ecology
Solid and Hazardous Waste Program
PO Box 47600
Olympia, WA 98504-7600
(206) 407-6753

WEST VIRGINIA

WV Division of Environmental Protection
Office of Waste Management
1356 Hansford Street
Charleston, WV 25301
(304) 558-5929

WISCONSIN

Department of Natural Resources
Bureau of Solid Waste Management
101 South Webster Street
Madison, WI 53707
(608) 266-1327

WYOMING

Department of Environmental Quality
Solid and Hazardous Waste Division
122 West 25th Street
Cheyenne, WY 82002
(307) 777-7752

TSCA, RCRA, and CERCLA Information Phone Lines

Toxic Substances Control Act (TSCA)
Assistance Information Hotline
(202) 554-1404

RCRA/CERCLA Hotline
(800) 424-9346
in the Washington, DC Metro Area
(703) 412-9810

POSTER SESSION PAPERS



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Effect of Dry and Moist Heating on the Formation of Lipid-protein Complexes in Soybean Products

E.A.Abd El-Motaal, H.E. Helmy, F.S. Taha and Z.E. Shoeb

Fats and Oils Department, National Research Centre, Dokki, Cairo, Egypt.

INTRODUCTION

Protein present in food may undergo great changes in the course of processing or storage. Many natural and processed foods contain lipid-protein complexes in which nonpolar and polar lipids are dispersed and often held by proteinaceous networks.

Lipid-protein interactions have been phenomena cited in the literature as important in biological processes and in food systems. They are of fundamental importance in food processing, and several characteristics of foods such as colour, odour, stability and texture are clearly affected by these interactions (1-5).

lipid-protein interaction forces may involve covalent bonds, hydrophobic or Van der Waal forces, electrostatic bonds and hydrogen bonds. Electrostatic and hydrophobic bonds are thought to be especially important in lipid-protein interactions (1, 6).

Under the high temperature, pressure, and mixing encountered during cooking and extraction processing, oxidation of the unsaturated lipids especially the polyunsaturated fatty acids may occur. Lipid hydroperoxides and their breakdown products such as aldehydes or ketones may then attack proteins to form covalent bonds. In addition to these reactions malonaldehyde, a common oxidative breakdown product of polyunsaturated fatty acids with three double bonds or more, was shown by Chio and Tappel (7) to cross-link amino acids by formation of a fluorescent 1-amino-3-iminopropene structure.

Soybean is an untapped source of both oil and protein. The world market is full of processed and fabricated foods derived from soybean, or novel food items fortified with soybean protein products. Therefore, it seemed worthwhile to investigate some of the factors that might affect the formation of lipid-protein complexes in the foods based on soybean during their processing and storage. In order to achieve this goal, it became clear that a study of the relation between soybean protein, soybean oil, and soybean gums is essential. This was accomplished by formulating model mixtures from the three above mentioned soybean ingredients, and treated them under conditions encountered during industrial processing of soybeans.

MATERIALS AND METHODS

MATERIALS:

In this study the materials were supplied by Al- Badrachin Factory (Cairo Company for Oils and soaps). These included : Crude soybean oil (CSO), gums (Gu), defatted soybean meal (SM) which was re-extracted with commercial hexane to reach residual oil content of 1 %, and soybean cake (SK), this is crushed soybean containing both the oil and protein.

From the above materials the following was prepared in the laboratory : Part of the SO was heated at 100°C in an oven to obtain oxidized crude soybean oil (OxCSO) with a peroxide value ranging between 119-126. Gums were separated from the oil after heating to obtain degummed oxidized soybean oil (DgOxCSO) Degumming was carried out according to the procedure of cousins et al.(8).

To study the formation of lipid-protein complex formation in soybeans, model mixtures were formulated as follows:

- I- 70g soybean meal (SM) + 30g crude soybean oil (CSO)
- II- 70g soybean meal (SM) + 30g oxidized crude soybean oil (OxCSO)
- III-70g soybean meal (SM) + 30g degummed oxidized soybean oil (DgOxCSO)
- IV-79g soybean meal (SM) + 3g gums (Gu)
- V- soybean cake (SK),acting as control

These model mixtures were well homogenized and together with the control were subjected to dry and moist heating as illustrated in Figures 1 and 2.

Dry heating (DH): The mixtures were placed in closed containers and subjected to dry heating in an oven at a temperature of 90 °C for periods of one ,two, and three days.

Moist heating (MH) : In this treatment water was added to reach a moisture content of 10 %, 30 %, and 50% to each of the four model mixtures as well as the control, the mixtures were carefully mixed using an electric mixer, then heated in an oven at a temperature of 100°C, for periods of one ,two, and three days.

Oil Extraction: At the end of each period of the above treatments, model mixtures I, II, III as well as the control were subjected to extraction with commercial hexane using a soxhlet apparatus . Model mixture IV was subjected to extraction with chloroform. The solvent was stripped using a rotary evaporator. The extracted oil and gums were kept in brown bottles in a refrigerator. The defatted meals resulting after extraction of oil was spread to dry ,then sieved to pass an 80 mesh screen ,and kept in closed glass containers for further analysis.

METHODS OF ANALYSIS

Lipid Analysis: The extracted oils from the treated mixtures as well as the control were analyzed for : free and bound lipids, peroxide value and spot test. While the gum extract was subjected to spectrophotometric analysis.

Determination of free and bound lipids (9) :About 5 grams of the treated soybean mixture was weighed accurately and subjected to a two step extraction. First extraction with n-hexane using a soxhlet apparatus, the oil resulting from this extraction was calculated on dry basis and referred to as free lipid (FL). The residue resulting after extraction with hexane was again subjected to extraction with water saturated butanol. This extract was referred to as bound lipid (BL).

Peroxide value (10):This was determined according to A.O.C.S. Official methods of analysis.

Spot test (11): The extracted oil sample was spotted on a filter paper, dipped in 0.1% aqueous solution of amido black 10B for 5 min., removed from the dye and washed with distilled water. The presence of protein was indicated by a dark spot on a white background.

Spectrophotometric analysis :The gums were analyzed spectrophotometrically as follows: The extracted gum samples as well as the untreated gum s (control)were dissolved in carbon tetrachloride to give 1 % (w / v)solution . the untreated gums was dissolved in the ratio of 1: 5 with carbon tetrachloride. The absorption spectra of the different samples were compared to that of control at wave lengths ranging from 300-700nm. using a Shimadzu UV-visible recording spectrophotometer, model UV 240.

Meal Analysis:

The meal resulting after oil extraction from mixtures I, II, III, IV, as well as soybean cake were analyzed for nitrogen and total protein using a semimicro kjeldahl procedure (12) ,

RESULTS AND DISCUSSION

Spot test showed that lipid-protein complexes are present in all the investigated mixtures except the three dry heated soybean cakes as well as the 10 % moisture heated for one day.

A. DRY HEATING OF SOYBEAN MIXTURES

The model mixtures formulated with the aim of studying lipid-protein complexes during soybean processing were subjected to dry heating. The oils extracted from the dry heated mixtures were analyzed to see the effect of dry heating on the formation of the lipid-protein complexes.

Peroxide value: Figure 3 shows the changes in the peroxide value (PV) in the peroxide values of the oils extracted from the treated mixtures together with the oil resulting from the soybean cake acting as control.

It is clear that no oxidation occurred in the case of the oil from soybean cake throughout the period of dry heating. This might be due to the naturally occurring antioxidants in the oil such as tocopherols, also the protein can act as an antioxidant during the oxidation of phospholipids and lipids, this is accomplished by the reaction of hydroperoxides with methionine resulting finally in methionine sulphone which has an antioxidant effect (15).

Crude soybean oil has a zero peroxide value, while oxidized soybean oil has a peroxide 119.0 PV, degummed oxidized soybean oil show a slight increase over the oxidized oil reaching 122.4 PV. An extra high PV 343.9 was observed for the oil resulting from mixture I after one day of dry heating, which was followed by a clear decrease after the second and third day of dry heating reaching 81.40 and 50.40 PV, respectively.

Peroxide values of both oils resulting from soybean mixtures IIDH AND IIIDH after 1, 2, and 3 days of heating show a decrease in PV 32.10 , 26.80, 15.90, respectively, and 48.30, 20.90 and 11.80, respectively.

Hydroperoxides are the initial products of oxidation and account for the majority of bound oxygen usually measured by peroxide value (16).

Pokorny et al. (17), reported that in mixtures of lipids and proteins the reaction course depends on the water content. In dry systems autoxidation of lipids proceeds slowly during an induction period but becomes very rapid in the subsequent stage. Low peroxide values in mixtures with proteins are caused by rapid destruction of hydroperoxides in contact with protein solution.

Again Pokorny et al. (18), stated that polar groups of oxidized lipids formed non-extractable compounds with protein more readily than polar groups of monoglycerides. The formation of these nonextractable compounds is due both to the interactions of proteins with hydroperoxides and with non-peroxidic oxidation products. The same authors and EL-Tarras et al.(19) concluded that the amount of these non-extractable lipids in protein-lipid systems was

proportional to the amount of peroxides decomposed during heating of the mixture.

2. Free and bound lipid content: The hexane extracted oils resulting from models I, II, and III as well as the control were examined for their free and bound lipid contents. Results are diagrammatically represented in Figures 4 and 5.

The control showed no appreciable change in either the free nor the bound lipid content on dry heating.

The soybean mixtures with crude soybean oil IDH showed a considerable increase in the free lipid content on the first day of heating followed by a decrease in the free lipid content on second and third days. The bound lipid content remained more or less the same at the beginning of the experiment and after day 1 and day 2 of dry heating ranging between 21-22%, after the third day it decreased to 18.2%.

The two mixtures containing the oxidized oil IIDH and IIIDH showed a decrease in the free lipid content by increasing the days of heating, meaning more complexation of lipid with protein took place, but unexpectedly this decrease in free lipid was not accompanied by a corresponding increase in the bound lipid content, mixture IIDH showed a decrease in bound lipid content with increasing time of heating, while IIIDH showed a slight increase in bound lipid content on first and second days of heating and decreased again on third day.

Pokorny et al. (20), discussing methods of analysis of bound and free lipids reported that methods of analysis should be selected, and that some lipids remain bound and cannot be split.

Perhaps water saturated butanol was not the suitable solvent in this extraction. The conclusion is free and bound lipids cannot be taken as a criteria for lipid-protein complexation in this case.

3. Spectrophotometric analysis: This was carried out on the chloroform extracted gums of model mixture IV as well as the untreated gums.

Figure 6 represents the absorption spectra of the chloroform extracts of soybean mixture IV during dry heating in addition to the untreated soybean gums (control). The samples under investigation were IVDH1, IVDH2, and IVDH3. The spectra show the effect of heating at 95°C on the soybean meal mixed with soybean gums for 1, 2, and 3 days. It is clear that there is a slight decrease in the optical densities of the spectra of the extracted gums with increasing time of heating. On the other hand, the optical densities of the spectrum of the untreated gums is higher than that of the treated gums within the soybean mixtures.

4. Nitrogen solubility and digestibility of the meal fraction: Figures 7 and 8 gives the nitrogen solubility % and digestibility % of the soybean meal samples resulting after extraction of the oil from the dry heated mixtures including : mixtures I, II, III, and IV ,as well as control V , the dry heating was carried out for 1, 2, and 3 days.

By looking at Figures 7 and 8 it can be observed that the highest decrease in both nitrogen solubility and digestibility always took place for mixture III, which is the soybean meal + degummed oxidized soybean oil. This result confirms that the gums act as an antioxidant and thus reduces the lipid-protein formation. This result is also in agreement with results of the peroxide value .

Least decrease in nitrogen solubility and digestibility was always noticed with the meal protein of mixtures I and IV. Mixture I contains the crude soybean oil . This result is also in agreement with the result of the peroxide value. The meal protein of mixture IV which contains the gums showed also little decrease in both the nitrogen solubility and digestibility perhaps because the gums are mainly lecithin.

B. MOIST HEATING OF SOYBEAN MIXTURES

After subjecting the model mixtures to moist heating, the extracted oils were analyzed for:

1. Peroxide value: The changes in the peroxide value of the extracted oils resulting from the soybean mixtures after moist heating, in addition to a control sample resulting from soybean cake are given in Figures 9 and 10.

Results reveal that oil from soybean cake containing 10 % moisture and heated at 100°C showed a peroxide value zero after 1, 2, and 3 days of heating.

Increasing the moisture content of all investigated mixtures to 30 and 50% moisture , and heating for 1, 2, and 3 days resulted in an increase in peroxide value. 50% moisture > 30% moisture > 10% moisture. Oil resulting from mixtures IMH and IIMH when heated for the third day behaved differently. There was a decrease in peroxide value with increasing the moisture content. For IMH peroxide values were 7.10, 6.00 and 4.00 for 10%, 30%, and 50% moisture ,respectively.

A sharp increase in the PV was noticed with the oil resulting from IMH, where the crude soybean oil had a zero PV which increased to 151.90, 172.40, and 192.20 when 10, 30, and 50% moisture were added during the first day , respectively. This again was followed by a noticeable decrease during the second

and third days. This result is similar to the result of dry heating the same mixture I. , where there was also a sharp increase in PV after the first day followed by a noticeable decrease in PV during the second and third days.

When comparing results of IIMH and IIIMH, the oil resulting from IIMH which was initially an oxidized oil in the mixture had a PV much less than the oils resulting from IIIDH which resulted from the mixture containing the degummed oxidized soybean oil. This result could be explained on the basis of the antioxidant effect of the gums or phospholipids.

In general , the results reveal that there is a clear relation between increasing the time of heating and the peroxide value. The PV decreases with increasing time of heating, this is similar to results of dry heating. This might be explained by the probable interaction between the added oxidized oils, as well as the autoxidation of the crude soybean oil and the protein to give lipid-protein complexes. Or perhaps the prolonged heating causes the hydroperoxides to be further into secondary oxidation products.

2. Free and bound lipid content: Figures 11 to 14 represent the changes in the free and bound lipids of the soybean mixtures during moist heating.

Slight changes have been observed in the free and bound lipid contents of all the control samples (VMH) during moist heating.

Moist heated mixtures of soybean meal mixed with crude soybean oil (IMH)at different moisture levels resulted in a decrease in the free oil content of the samples with increasing days of heating. This decrease in free lipid content was not accompanied by an increase in the bound lipid content. Differences in values of bound lipids are so small that they cannot be discussed. The highest content of bound lipids 24% was reached after 3 days of moist of mixture IMH with 50% moisture.

Changes in the free and bound lipid contents of mixtures oxidized oil IIMH and degummed oxidized oil IIIMH at different moisture levels show the same pattern of decrease in free lipid content with increasing the time of heating. For mixture IIMH there is a decrease in the bound lipid content on the first and second days of heating, followed by a slight increase on the third day for the sample containing 50% moisture. Mixtures IIIMH with 10 and 30% moisture show slightly higher bound lipid contents ca. 24% by the third day of heating when compared 22% before treatment. Mixture IIIMH WITH 50% moisture behaved as expected showing an increase in bound lipid content with increasing time of heating and reaches the highest bound value for all the examined samples 29%.

2. Spectrophotometric analysis: Figure 15a-15c give the absorption spectra of the chloroform extracts of the moist heated soybean mixtures mixed with soybean

gums (IVMH) in addition to the untreated gums as control . The spectra show the effect of heating at 100 C of soybean meal mixed with soybean gums containing 10, 30, and 50% moisture within a period of three days. Decrease in the optical densities of the spectra was observed after the first and second day heating by increasing the added moisture. After the third day, there was no clear change in the optical densities of the spectra of the extracted gums.

Karel (1), reported that the attraction between non-polar groups was greatly increased in aqueous media where they were pushed together by water ,where the water- interaction by hydrogen bonding was much stronger than interaction between water and the non-polar groups. This phenomena is known as" Hydrophobic bonding".

4. Nitrogen solubility and digestibility of the meal fraction: Nitrogen solubility and digestibility percents as well as the protein contents of the meals resulting from the moist heated soybean mixtures for 1, 2, and 3 days with 10 , 30, and 50% moisture are diagrammatically represented in Figures 16-21.

It is clear that the moist heating caused more reduction in the nitrogen solubility, and digestibility of the meal samples resulting after the extraction of the oil from the treated mixtures, than the same meal samples after dry heating.

It can also be observed that increasing the moisture content causes a concomitant decrease in both the nitrogen solubility and digestibility. This is in agreement with results of the peroxide values.

Results of both nitrogen solubility and digestibility are in agreement. They both show that the protein was damaged . Increasing the time of heating also increases the damage to the protein as manifested by the decrease in nitrogen solubility and digestibility.

Highest decrease in solubility reached 66% for mixture IIMH at 50% moisture after the first heating day. The least decrease in nitrogen solubility was 11% for mixture IIIMH at 30% moisture after 2 days heating, when compared to the corresponding cake.

Comparing meal of mixturesIIMH and IIIMH, results reveal that contrary to dry heating the protein in IIMH was more damaged than the protein in IIIMH, although mixtureIIMH contains oxidized soybean oil while mixture IIIMH contains degummed oxidized soybean oil.

Piroska (21,22) reported on the effect of heat on extracted sunflower meal ,and found that moist heat treatment at 120 C for 10-20 min. decreased protein solubility, increased denaturation of protein and caused loss in methionine, cystine, leucine, tyrosine, and serine. The nutritive value and digestibility were also reduced.

CONCLUSION

Thus it can be concluded from the above study that:

Increasing the moisture content increase the liability of lipid-protein complexation.

Prolonged heating also causes more lipid-protein formation

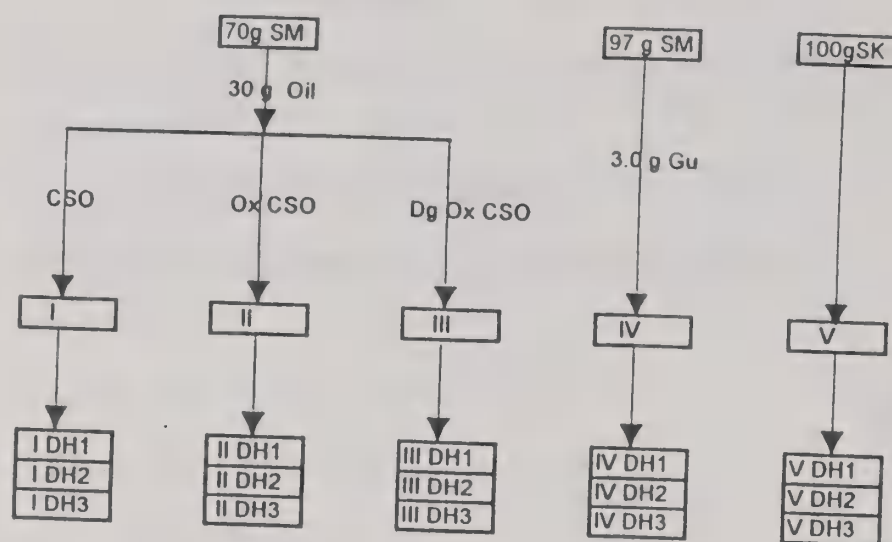
Factors that increase lipid-protein formation causes decreases in protein quality as indicated by decrease in nitrogen solubility and digestibility.

In general the presence of the gums in the oxidized oil causes less lipid-protein formation as indicated by peroxide value.

REFERENCES:

1. Karel, M., J. Food Science : 38, 756, 1973.
2. Hoseney, R. and Finney, K.F., Baker's Digest : 45, 30, 1971.
3. Schmidt, D.G., Buchhein, W., and Koops, N., Neth. Milk Dairy J. : 25, 200, 1971.
4. Wu, L.C. and Bates, R.P., J. Food Science : 38, 783, 1973.
5. Wu, L.C. and Bates, R.P., J. Food Science : 40, 160, 1975.
6. Chapman, D., Lipids : 4, 751, 1969.
7. Chio, K. and Tappel, A., Biochem. : 8, 2821, 1969.
8. Cousins, E.R., Fore, S.P., Janseen, H.J., and Fenge, R.O., J. A.O.C.S. : 30, 9, 1953.
9. Bekes, F., Zawistowska, U., and Bushuk, W., Cereal Chem. : 60, 367, 1983.
10. A.O.A.C., "Association of Official Agricultural Chemists", Official Methods of Analysis, Washington D.C., 13th ed., 1980.
11. Hoseney, R.C., Pomeranz, Y., and Finney, K.F., Cereal Chem. : 47, 153, 1970.
12. Clark, E.P., "Semimicro Quantitative Organic Analysis", Academic Press, N.Y., 1943.
13. Lyman, C.M., Chang, W.Y., and Couch, J.R., J. Nutrition: 49, 679, 1953.
14. Kanasawa, K., Ashida, H., and Nataka, M., J. Food Science : 52, 475, 1987.
15. Pokorny, J., Smidrkalova, E., Zwain, H., and Janicek, G., Nahrung : 19, 635, 1975.
16. Nilov, V.I., Ogorodnik, S.T., and Kharchova, P., Nauk-Virob zb. : 31, 40, 1967.
17. Pokorny, J., Smidrkalva, E., Zwain, H., and Janicek, G., Nahrung : 20, 707, 1976.

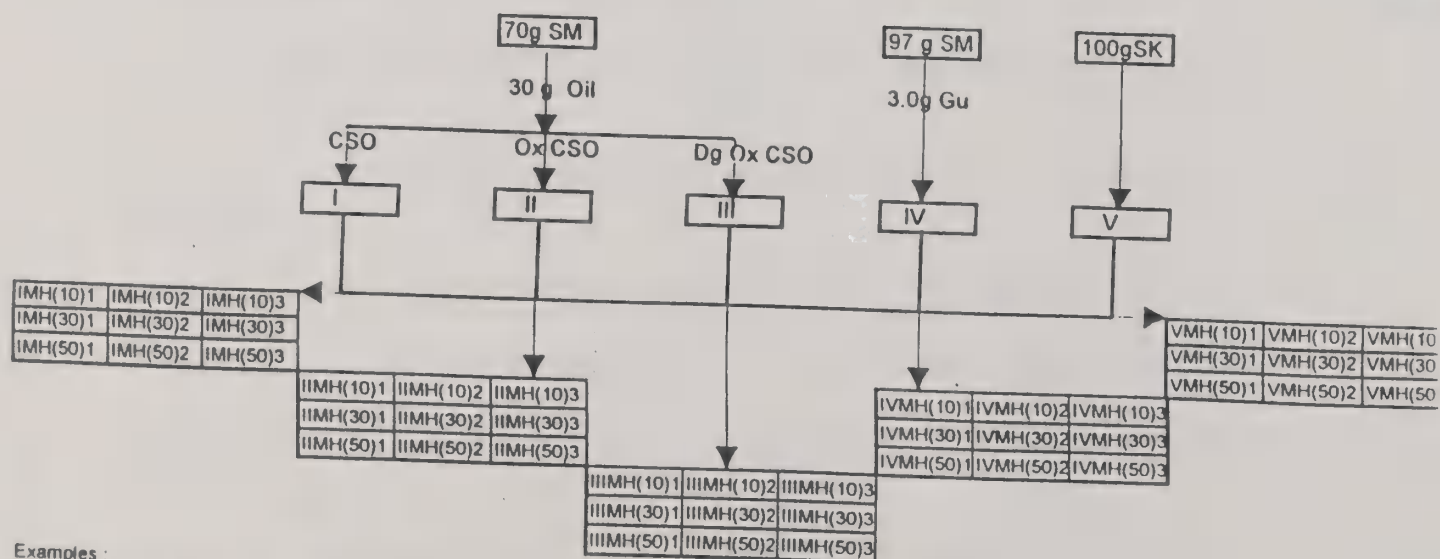
19. El-Tarras, M.F. , Pokorny, J., and Janicek, G., Nahrung : 15, 663, 1971.
20. Pokorny , J., Janicek, G., Davideh , J., Zeszyty Problemowe Postepow Nauk Rolniczych : 167, 155, 1975.
21. Pirooska,L.H., Olaj,Szappan, Kozmet.: 22, 9, 1973.
- 22.Pirooska, L.H., Tagunsber., Akad. Landwirtschaftswiss, DDR.: 124, 171, 1974.



Examples :

II DH 2 : Model mixtures II treated by dry heating for 2days .

Figure (1) : Schematic Representation of the Treated Soybean Mixtures During Dry Heating .



Examples :

IIMH(50)3 : Model mixture II treated moist heating ;

VMH(30)2 : model mixture V treated moist heating with 30% water for 2days .

Figure (2) : Schematic Representation of the Treated Soybean Mixtures During Moist Heating .

Figure (5) : Changes in the Peroxide Value of the Extracted Soybean Oil Samples from Soybean Mixtures During Dry Heating

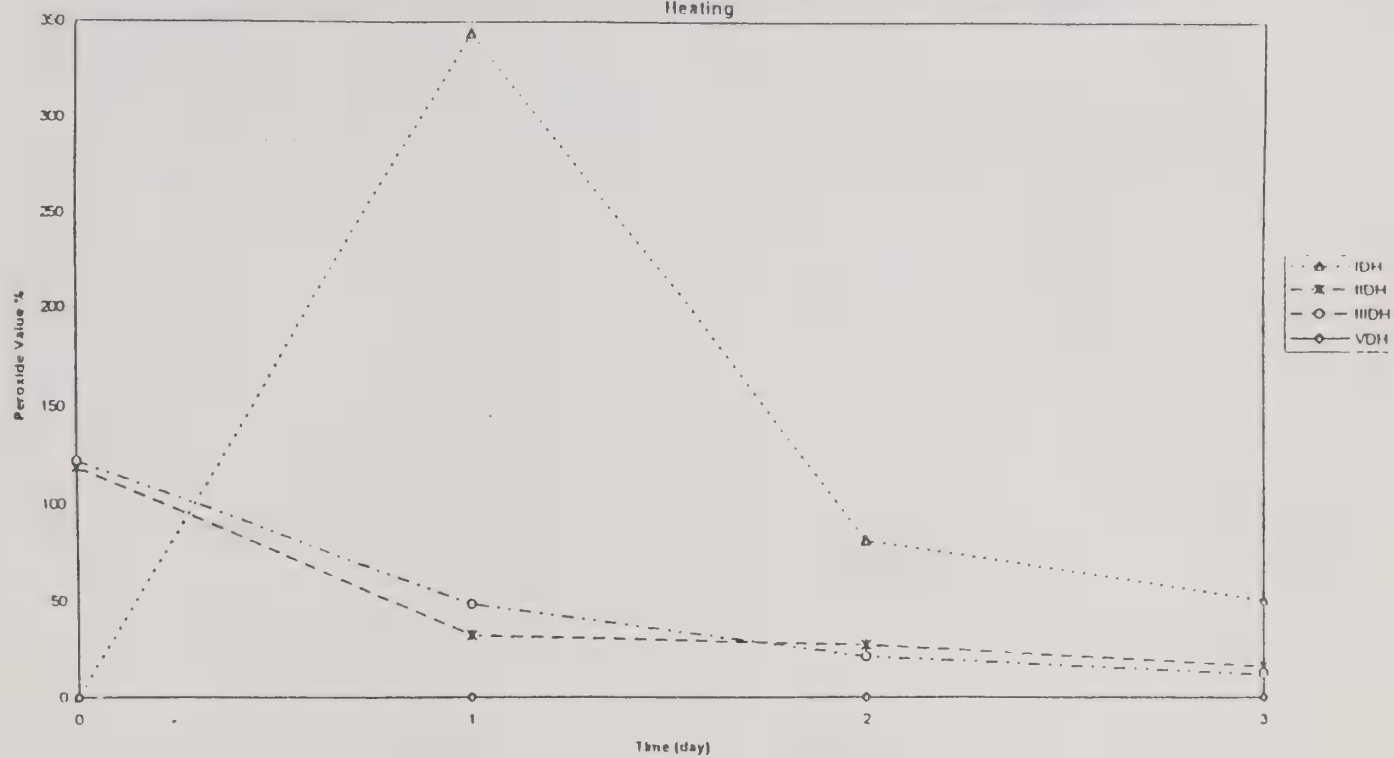


Figure (4) : Changes in the Free Lipid Content of Treated Soybean Mixtures During Dry Heating .

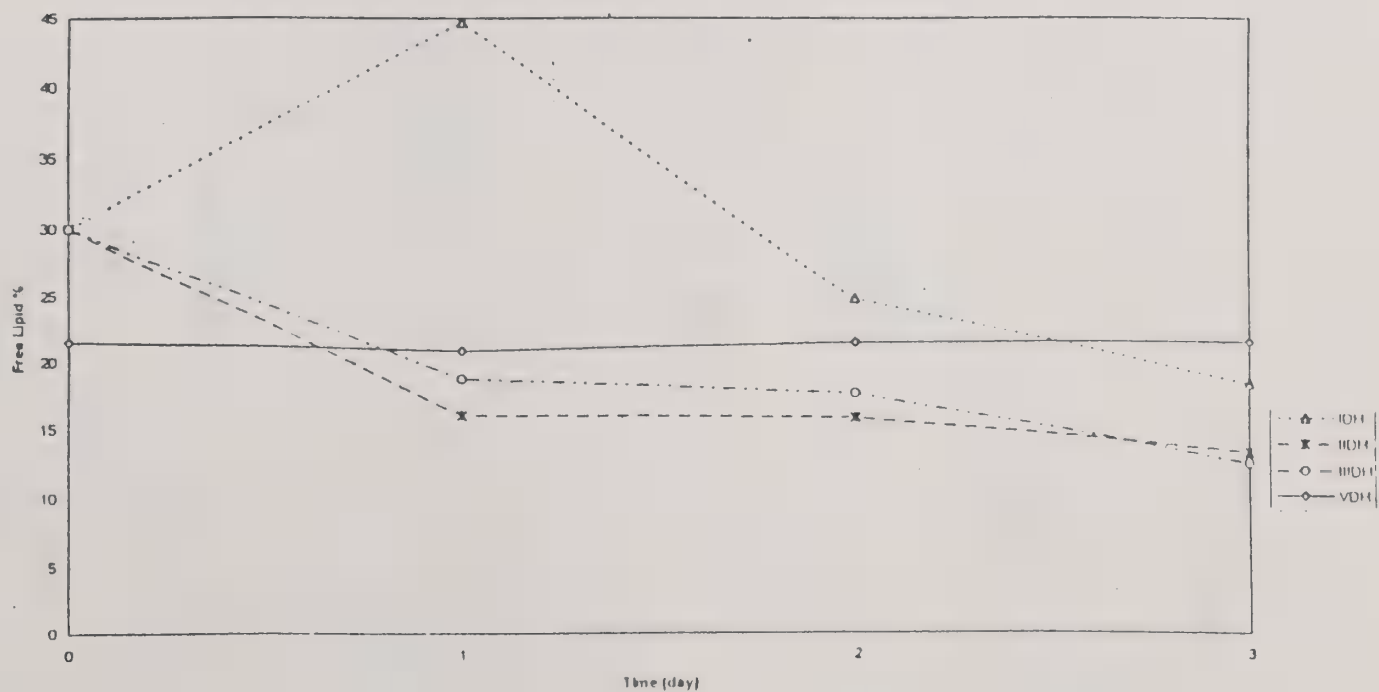


Figure (5) Changes in the Bound Lipid Content of Treated Soybean Mixtures During Dry Heating.

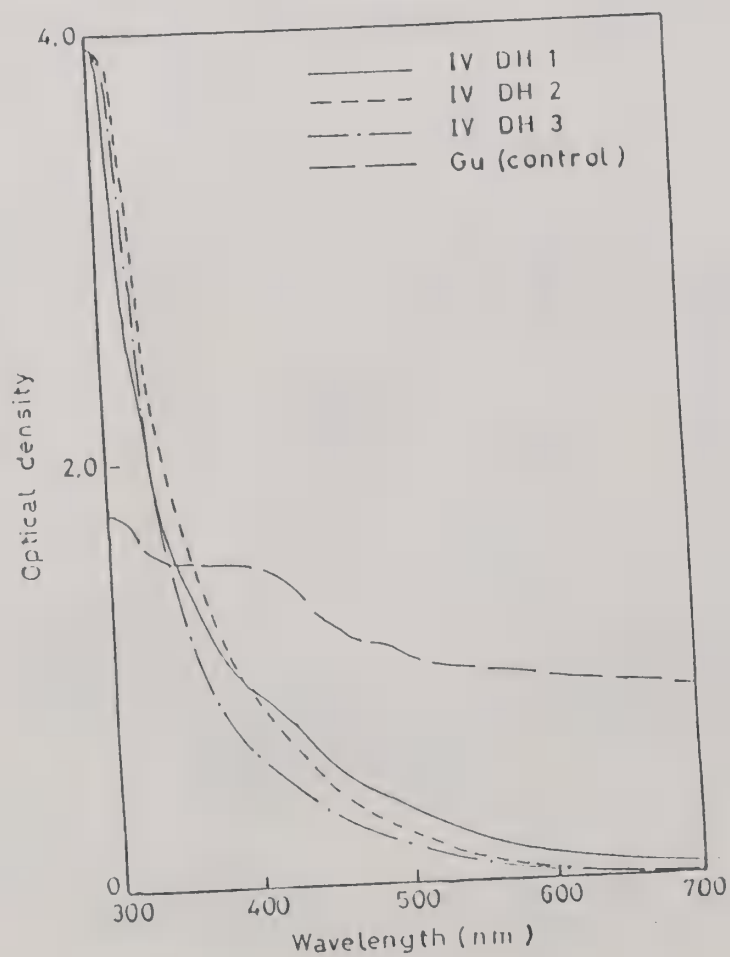
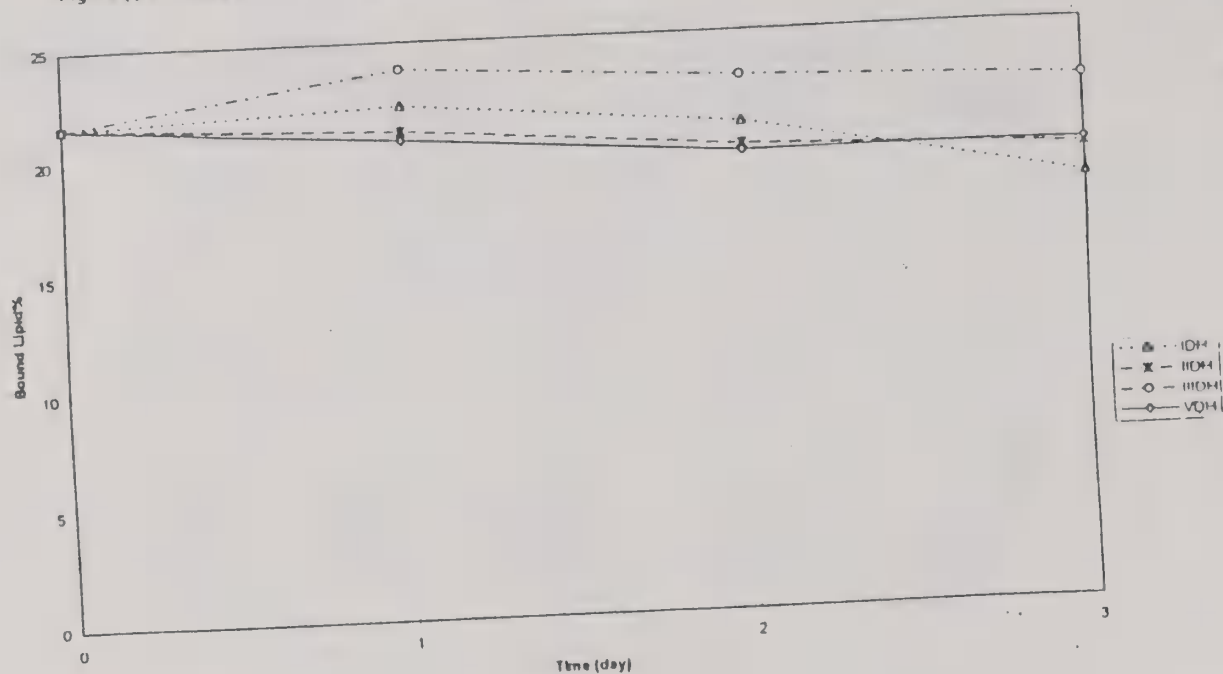


Fig. (6) : Absorption Spectra of Gum Extracts from Soybean Mixtures During Dry Heating, and the Untreated Soybean Gums (Gu).

Figure (7) :Changes In the Nitrogen Solubility of Treated Soybean Meal During Dry Heating .

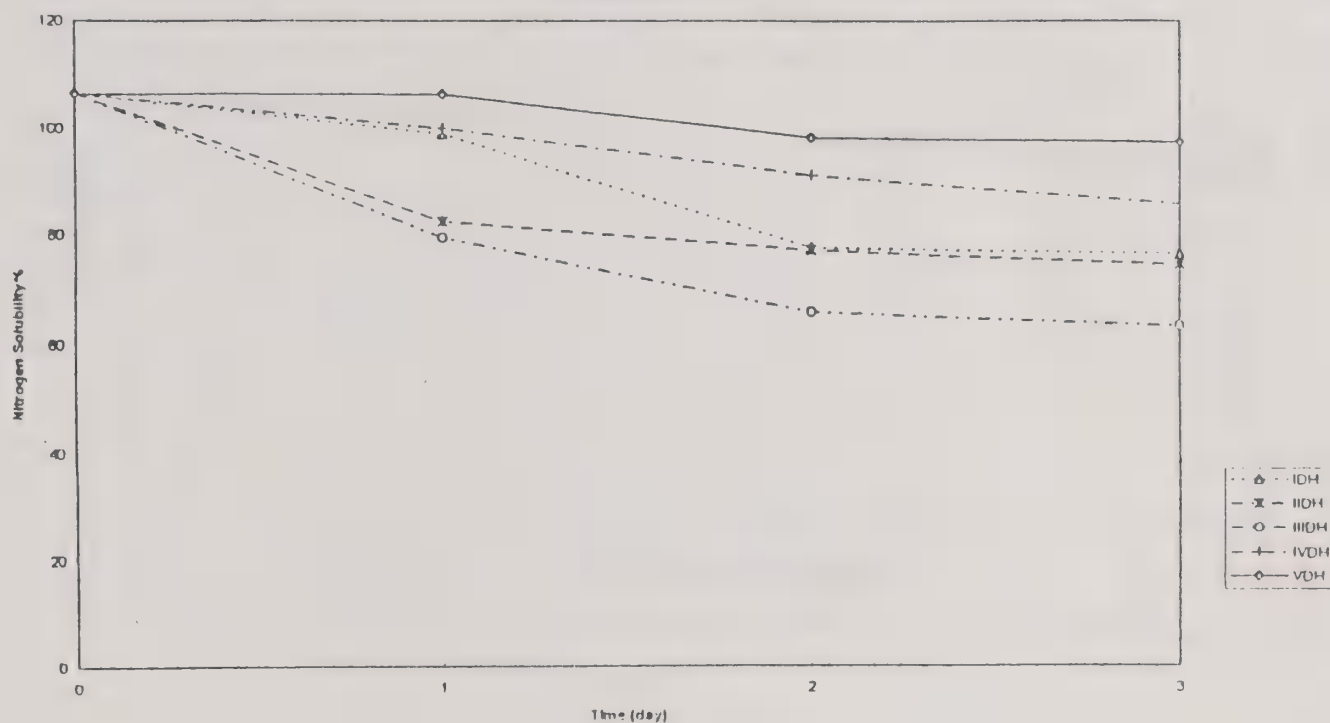


Figure (8) :Changes In the Digestibility of Treated Soybean Meal During Dry Heating .

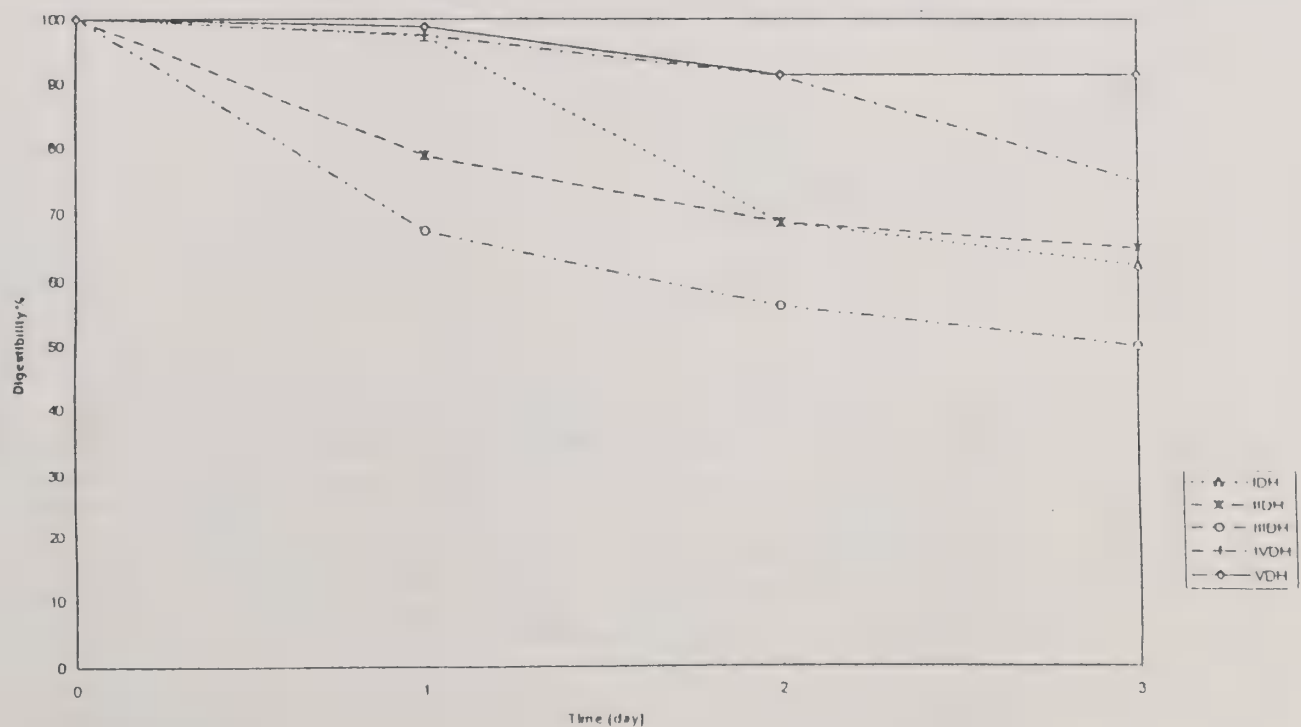
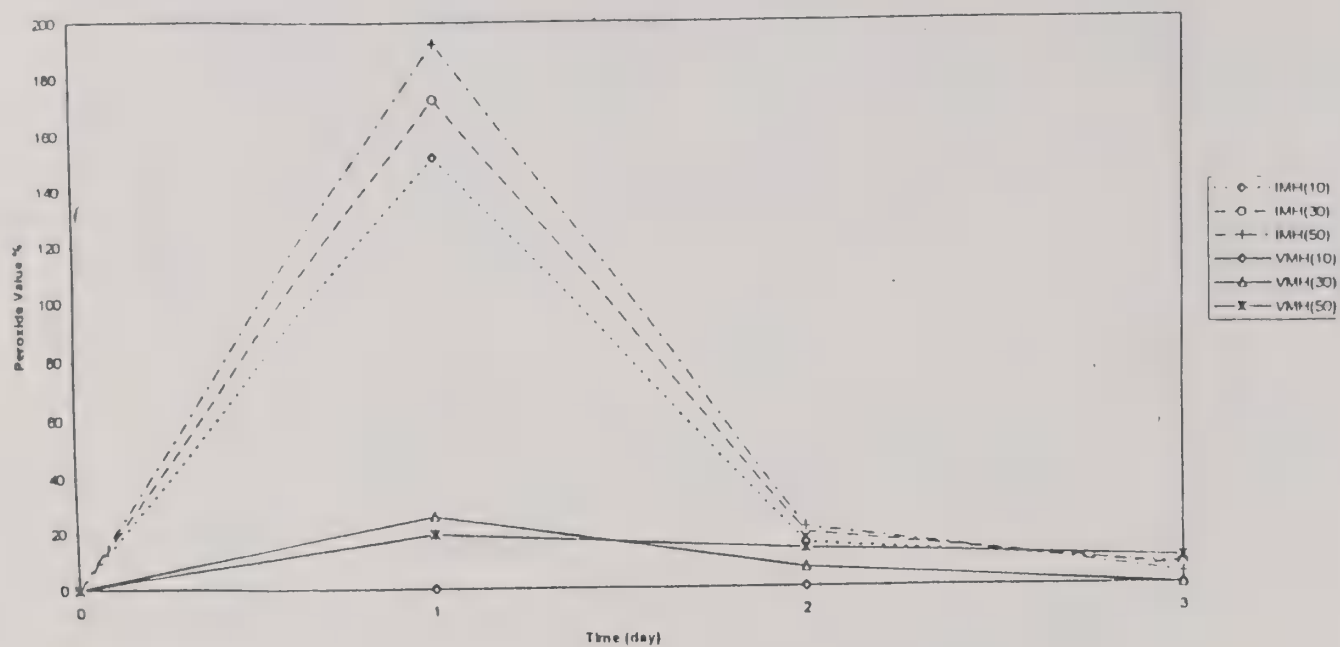


Figure (9): Changes In the Peroxide Value of the Extracted Soybean Oil from Soybean Mixtures (IMH&VMH) During Moist Heating .



Figure(10): Changes In the Peroxide Value of the Extracted Soybean Oil Samples from Soybean Mixtures (IIMH&IIIMH) During Moist Heating .

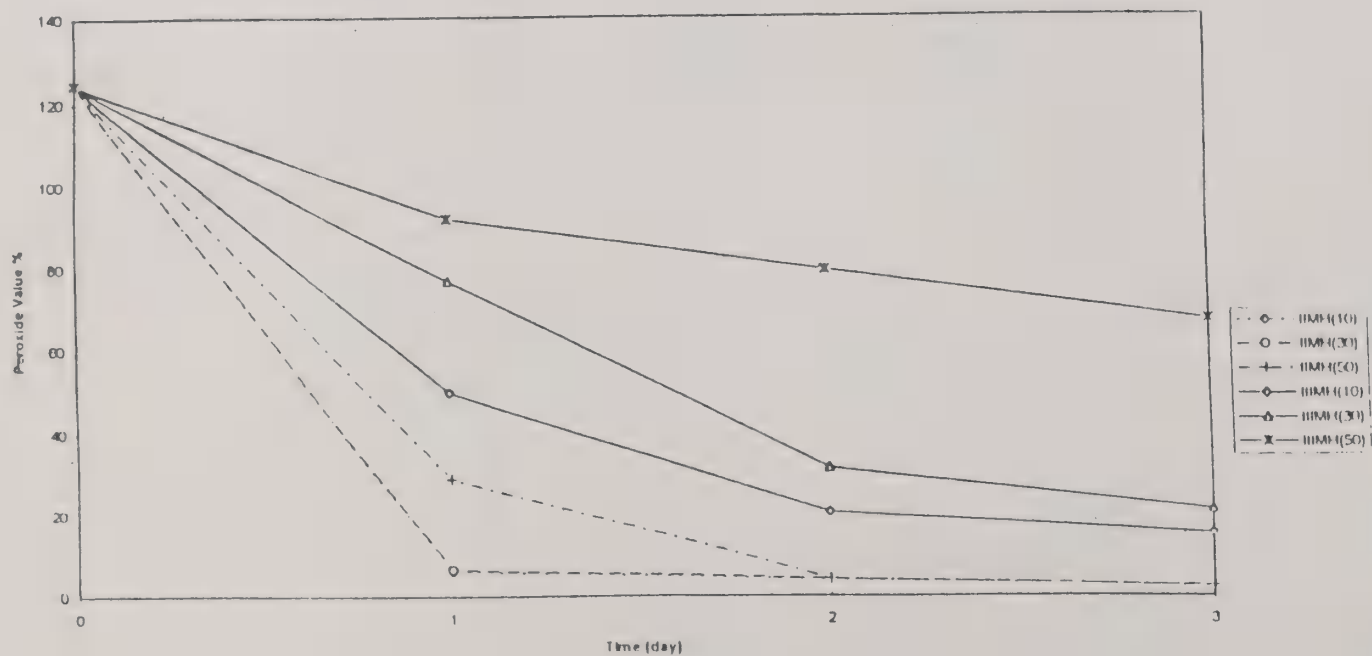
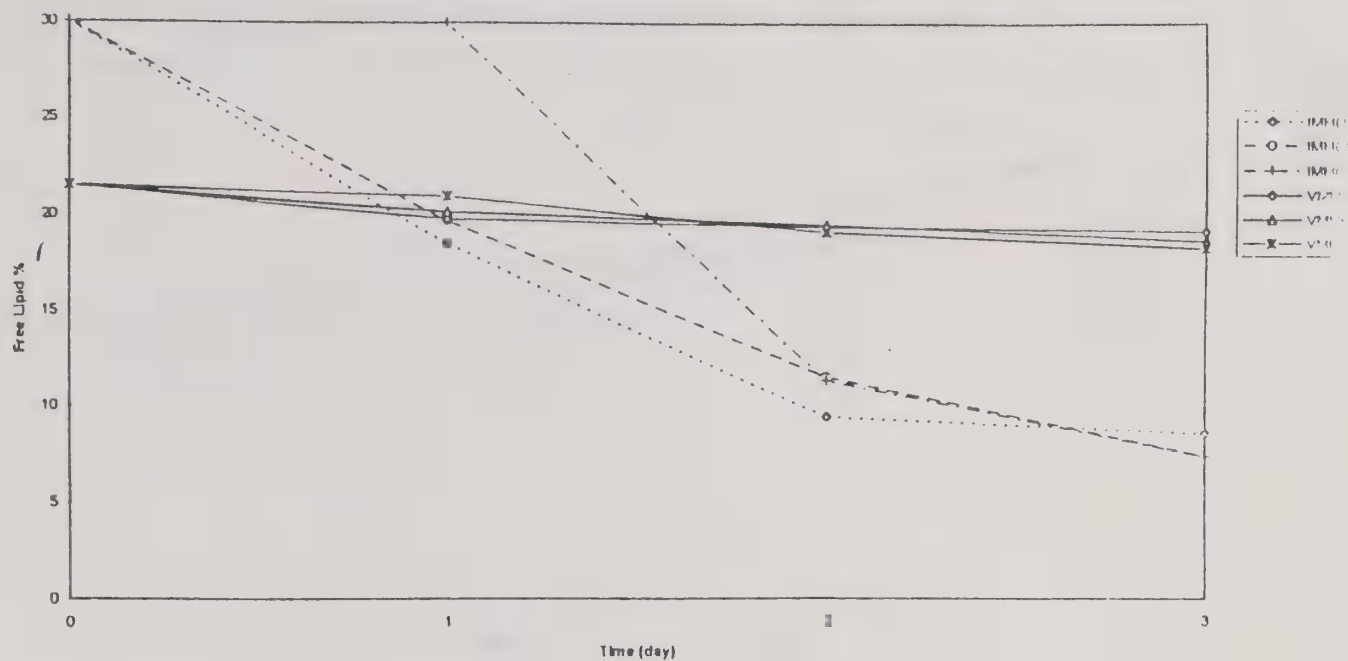


Figure (1) : Changes in the Free Lipid Content of Treated Soybean Mixtures (IMH&VMH) During Moist Heating .



Figure(2) : Changes in the Bound Lipid Content of Treated Soybean Mixtures (IMH&VMH) During Moist Heating .

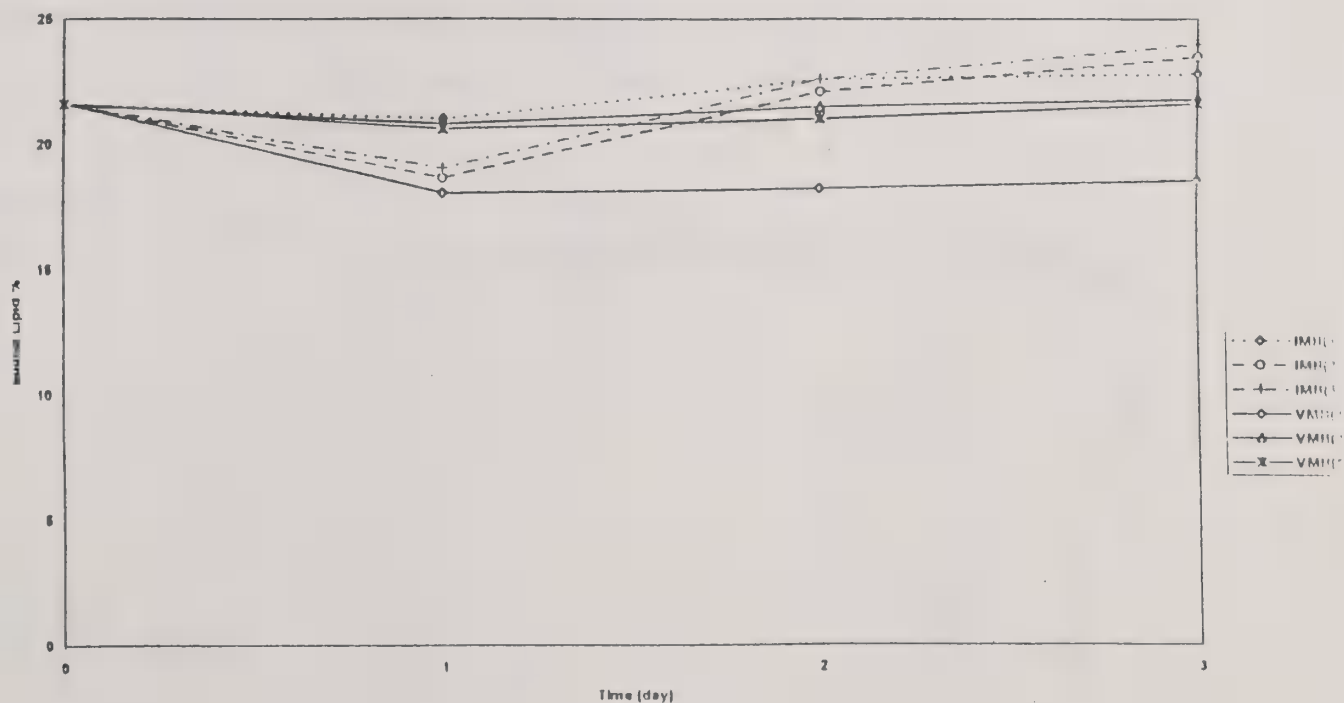
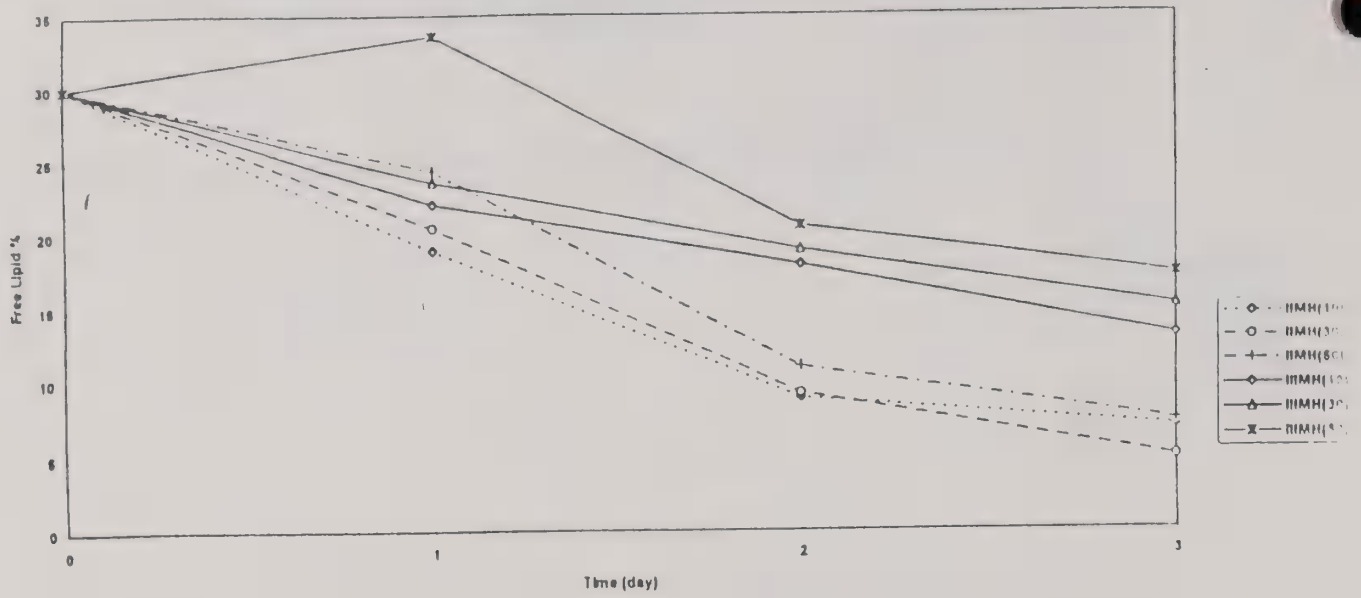
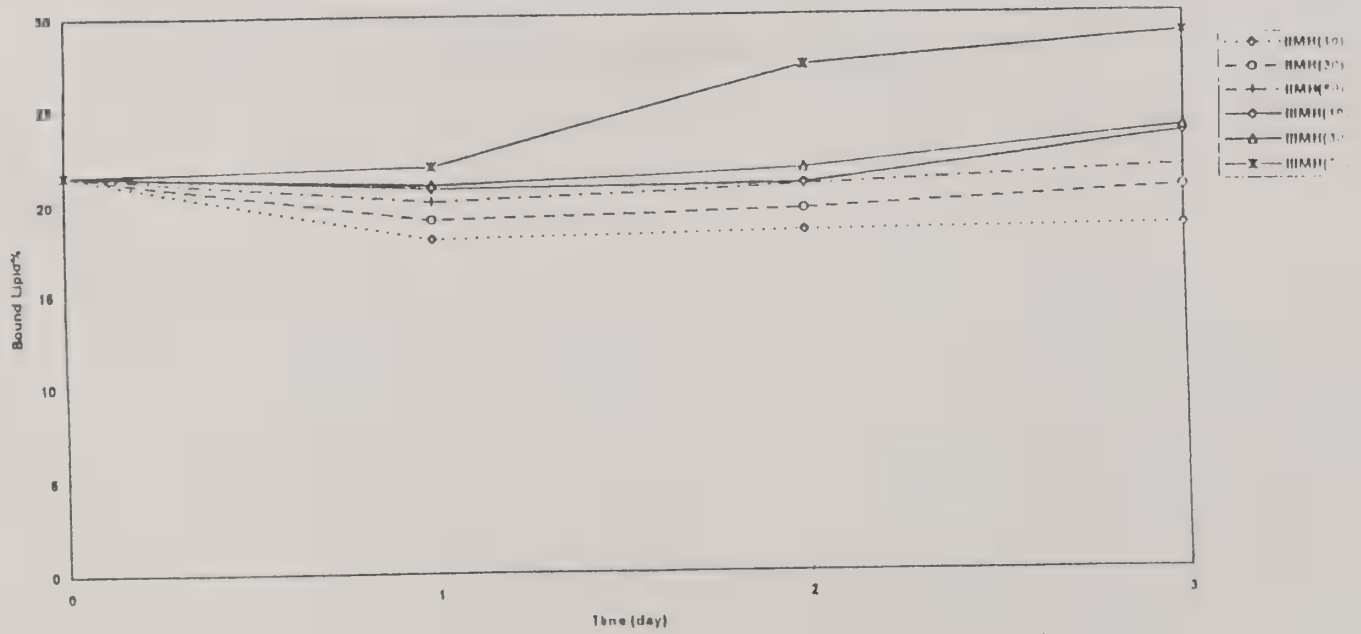


Figure (13) : Changes in the Free Lipid Content of Treated Soybean Mixtures(IIMH&IIMH) During Moist Heating .



Figure(14) : Changes in the Bound Lipid Content of Treated Soybean Mixtures(IIMH&IIMH) During Moist Heating .



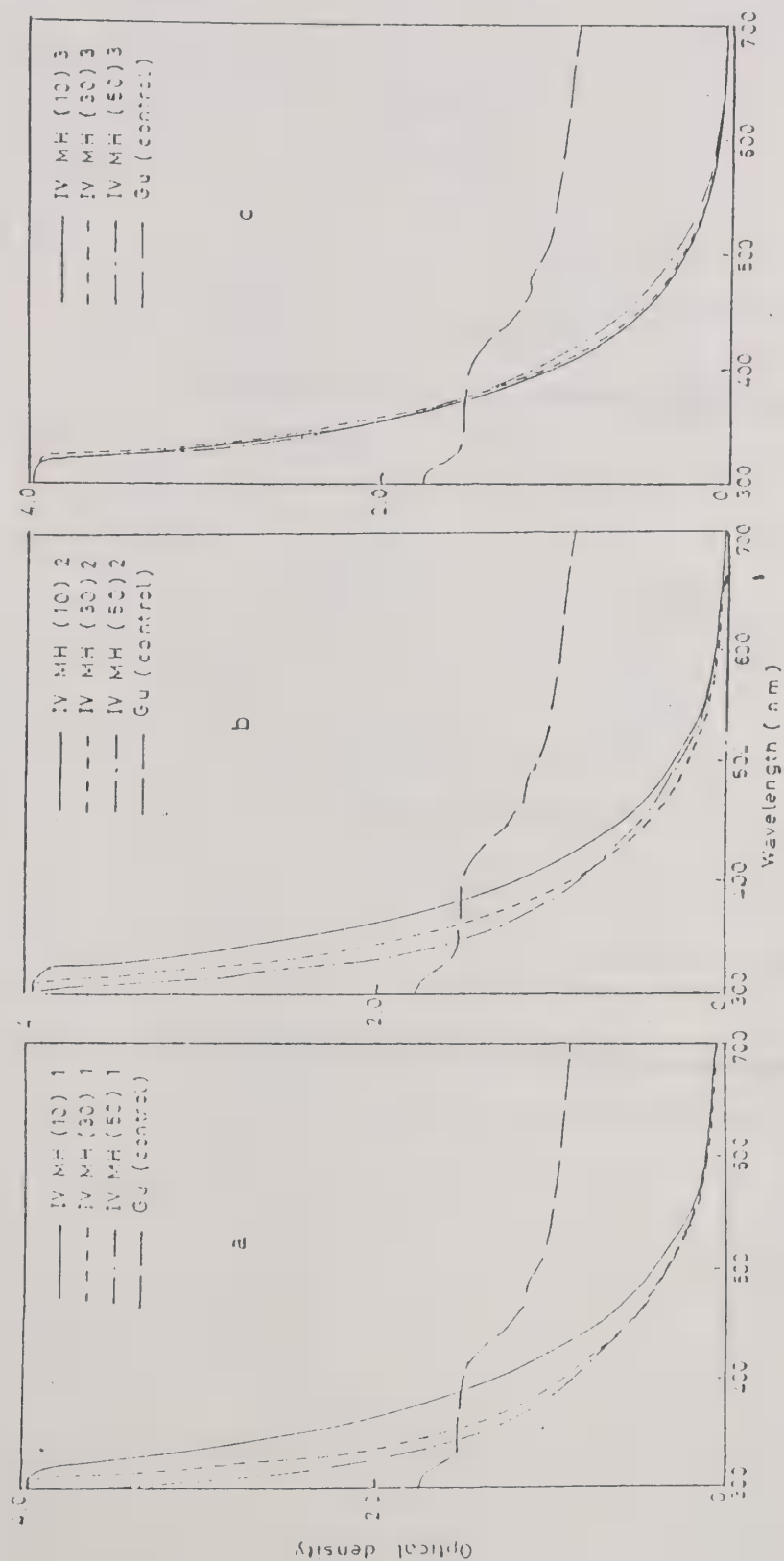


Fig. (15): Absorption Spectra of Gum's Extracts from Soybean Mixtures During Moist Heating
(a. IV MH 1, b. IV MH 2 and c. IV MH 3) and Untreated Soybean Gums (Gu)

Figure (16): Changes in the Nitrogen Solubility of Treated Soybean Meal Samples (IMH&VMH) During Moist Heating .

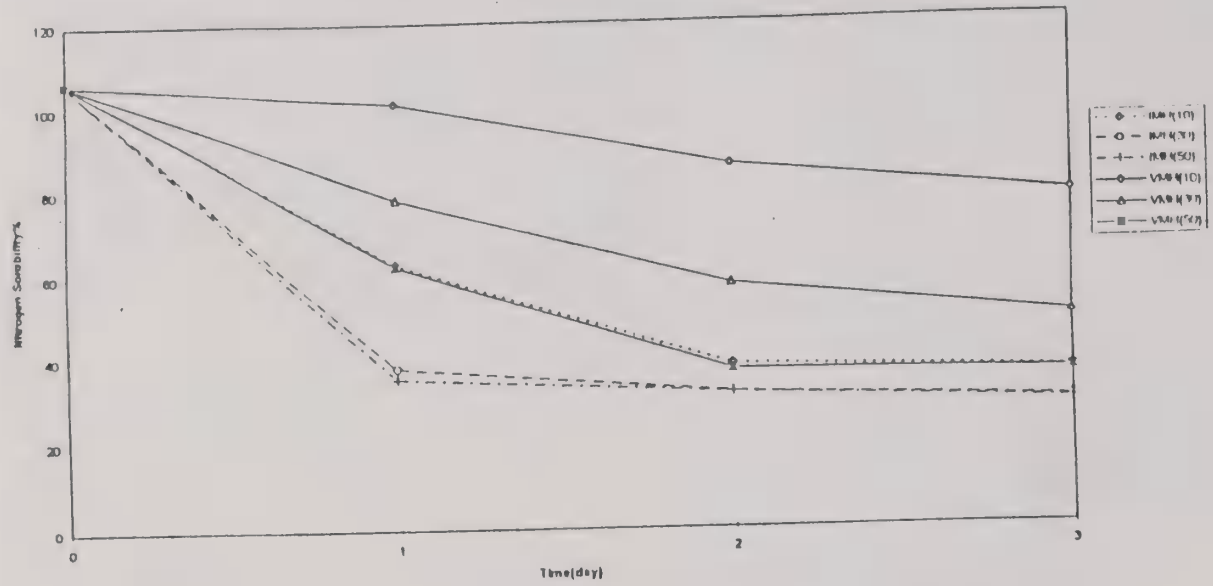


Figure (17): Changes in the Nitrogen Solubility of Treated Soybean Meal Samples (IIMH&IIIMH) During Moist Heating .

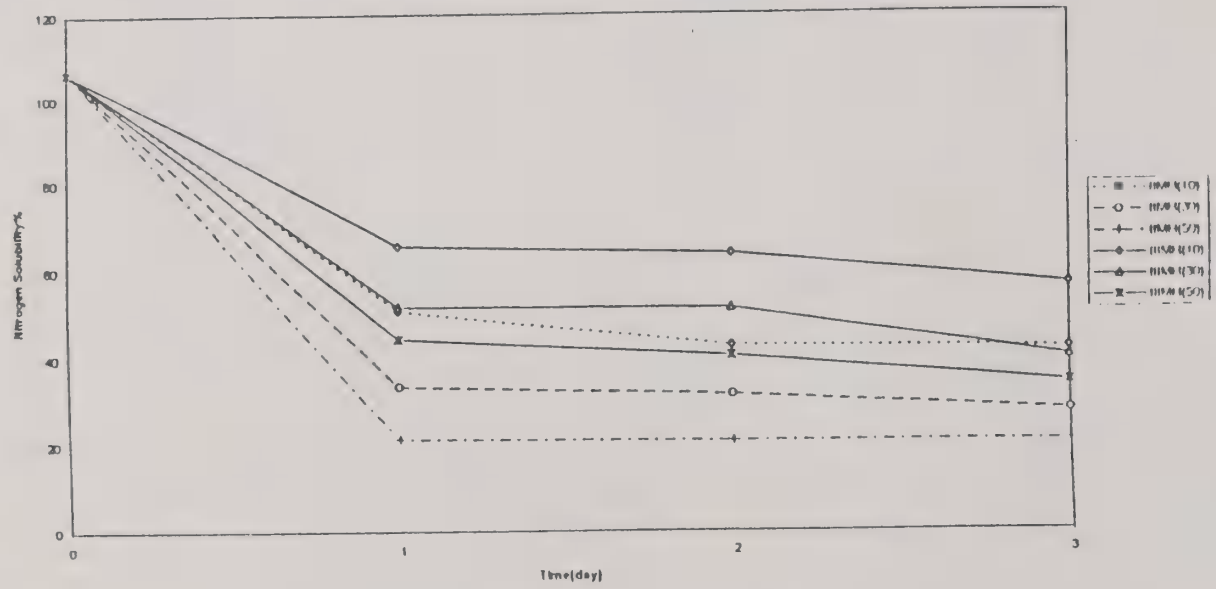


Figure (18): Changes in the Nitrogen Solubility of Treated Soybean Meal Samples (IVMH) During Moist Heating .

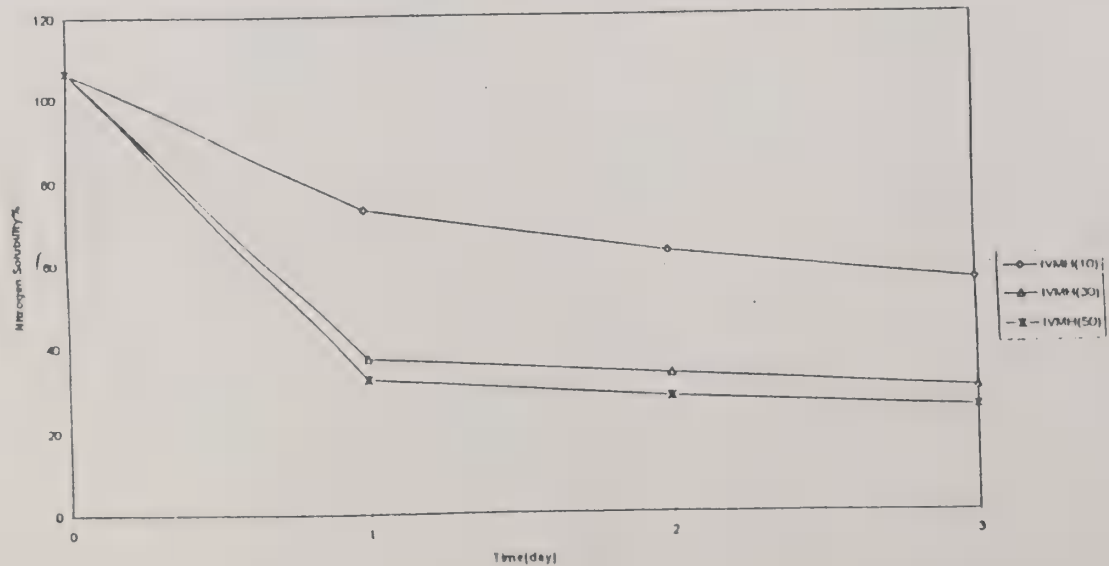


Figure (19) : Changes in the Digestibility of Treated Soybean Meal Samples (IMH&VMH) During Moist Heating .

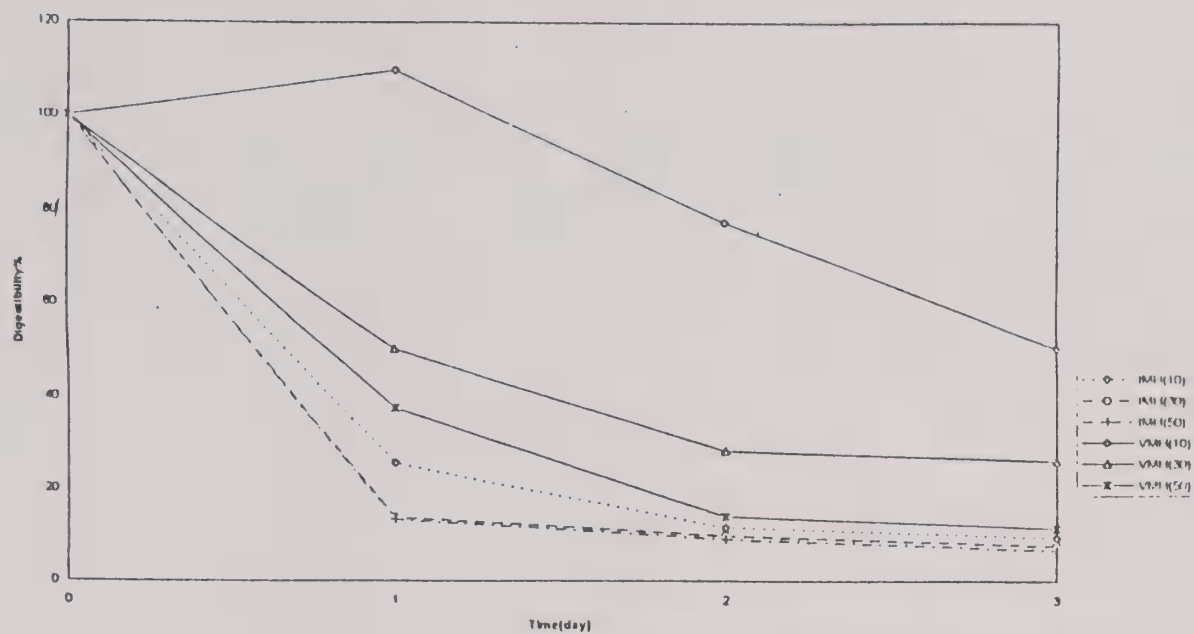


Figure (20) : Changes in the Digestibility of Treated Soybean Meal Samples (IIMH&IIMH) During Moist Heating .

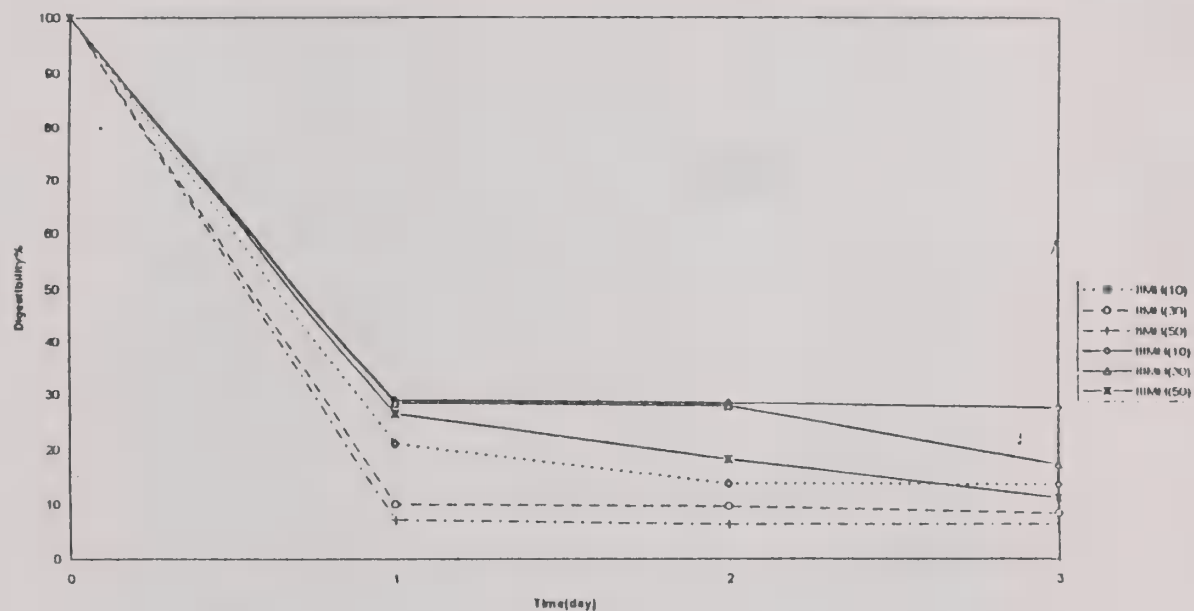
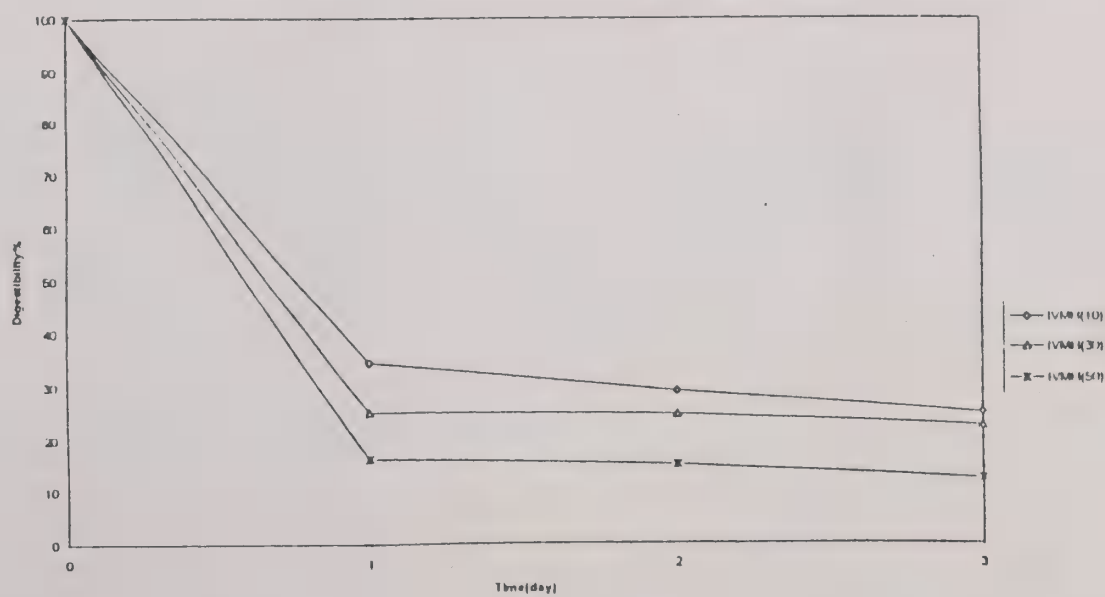


Figure (21) : Changes in the Digestibility of Treated Soybean Meal Samples (IVMH) During Moist Heating .



*Effect of Autoclaving and Storage on the Formation
of Lipid-Protein Complexes in Soybean Products*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Effect of Autoclaving and Storage on the Formation of Lipid-protein Complexes in Soybean Products

H.E.Helmy, E.A.Abd EL-Motaal,Z.E.Shoeb, and F.S.Taha

Fats and Oils Department, National Research Centre,Dokki,Cairo, Egypt.

INTRODUCTION

Lipid-protein interactions are of fundamental importance in food processing , because they affect food characteristics such as colour, odour, stability and texture, and also affects its nutritional value (1-3).

Under the high temperature, pressure, and mixing encountered, cooking and extraction processing, oxidation of the unsaturated lipids, especially the polyunsaturated fatty acids , may occur. Lipid hydroperoxides or their breakdown products such as aldehydes or ketones may then attack proteins to form covalent bonds. In addition to these reactions, malonaldehyde , a common oxidative breakdown product of polyunsaturated fatty acids with three double bond or more was reported to cross-link amino acids (4).

Protein- lipid interaction forces may involve covalent bonds, hydrophobic or Van der Waals forces, electrostatic bonds, and hydrogen bonds. Electrostatic and hydrophobic bonds are thought to be especially important in lipid-protein interactions. Van der Waals may occur between nonpolar lipid chains and nonpolar amino acid side chains in proteins. In the presence of water such interaction will be strengthened by hydrophobic bonding . Electrostatic binding may occur between positively charged protein residues and ionized fatty acids or negatively charged phosphate groups of phospholipids. Similarly positively charged phosphatidyl ethanolamine and negatively charged protein residues may also interact. Hydrogen bonding involving the hydroxyl groups of fatty acids , di and monoglycerides,or the head groups of phosphatidylethanolamine and phosphatidylserine, may occur with carbonyl groups of protein (2,5,6,).

The effect of dry and moist heating (conditioned similar to that encountered during soybean processing) on the formation of lipid-protein complexes was studied in a previous paper. Soybeans might be subjected to conditions similar to autoclaving during the processing of many products . Soybean products are subject to storage for different periods of time.

Therefore it seemed worthwhile to study the effect of autoclaving and storage on the formation of lipid-protein complexes in soybean products.

MATERIALS AND METHODS

MATERIALS:

Crude soybean oil (CSO), soybean gums (Gu), defatted soybean meal (SM), as well as soybean cake (SK) were kindly supplied by Al- Badrachin factory- Cairo Company for Oils and Soap. SM was re-extracted with hexane to give a meal with residual oil less than 1%. soybean cake was the crushed seeds containing both the oil and meal.

Part of the CSO and part of the Gu were heated at 100 °C in an oven to obtain an oxidized crude soybean oil (OxCSO) with a peroxide value ranging between 119-126. Gums (phosphatides) were separated from CSO after heating to obtain degummed oxidized crude soybean oil (Dg Ox CSO) according to the procedure of Cousins et al (7).

To study the formation of lipid-protein complexes, the following model mixtures were formulated:

- I- 70g soybean meal (SM) + 30g crude soybean oil (CSO)
- II- 70g soybean meal (SM) + 30g oxidized crude soybean oil (OxCSO)
- III- 70g soybean meal (SM) + 30g oxidized degummed soybean oil (Dg OxCSO)
- IV- 97g soybean meal (SM) + 3g gums (Gu)
- V- soybean cake (SK) which acts as control.

The four formulated mixtures as well as the control were carefully mixed to give a homogenous mixture with a fine texture. They were all subjected to autoclaving (Au) and storage (St).

Autoclaving : In this treatment the four model mixtures as well as the control were inserted in an autoclave at a temperature of 150 °C, and pressure of 1.8 atmosphere for a period of two hours.

Storage: the four model mixtures and the control were subjected to:

- 1- Storage at room temperature (RSt) for 1, 3, 5, and 8 weeks
- 2-Freeze storage (FSt) - freezing in a deep freezer for one week then stored at room temperature for periods of 1, 3, 5, and 8 weeks.

The above mentioned treatments are illustrated in Figures 1 and 2.

Oil Extraction: At the end of each period of autoclaving and storage the model mixtures I, II, III, and the control V were taken and subjected to extraction with commercial hexane using a soxhlet apparatus. Model mixture IV was subjected to extraction with chloroform.

The miscella resulting from each sample was dried with anhydrous sodium sulphate, the solvent then stripped at 45 C using a rotary evaporator. The extracted oils and gums were kept in brown bottles for further investigation.

The defatted residues of the four model mixtures and that of cake were spread to dry then ground and sieved to pass an 80 mesh screen and kept in closed glass containers in a refrigerator for further analysis. These residues constitute the meal fraction.

METHODS OF ANALYSIS

Lipid Analysis

Hexane extracts of the different treated model mixtures as well as the soybean cake were subjected to lipid analysis which comprises : free and bound lipids, peroxide value, spot test. While the chloroform extract was subjected to spectrophotometric analysis.

Determination of free and bound lipids (8)

About five grams of the treated soybean mixtures were weighed accurately and subjected to two step extraction procedure. First extraction with n-hexane using a soxhlet apparatus. The lipid content resulting from first extraction was calculated on dry basis and referred to as Free lipids (FL). Second extraction : The residue of soybean meal after hexane extraction was again subjected to extraction with water saturated butanol. The water saturated butanol lipid extract is referred to as bound lipids (BL).

Peroxide value (9) This was determined according to A.O.C.S. Official methods of analysis.

Spot test (1) The extracted oil sample was spotted on a filter paper, dipped in 0.1% aqueous solution of amido black 10B for 5 min., removed from the dye and washed with distilled water. The presence of protein was indicated by a dark spot on a white background.

Spectrophotometric analysis The gums were analysed spectrophotometrically as follows : The extracted gum samples as well as the untreated gums (control) were dissolved in carbon tetrachloride to give 1% (w/v) solution. The untreated gums were dissolved in the ratio of 1:5 (v/v) with carbon tetrachloride. The absorption spectra of the different samples were compared to that of the control at wave lengths ranging from 300-700 nm using a Shimadzu UV-visible recording spectrophotometer, model UV 240.

Meal Analysis

Soybean meal residues resulting from treated mixtures I, II, III, and IV as well as soybean cake were analysed for nitrogen and total protein using a semimicro kjeldahl procedure (10). Protein was calculated as Nx 6.25. Nitrogen solubility was carried out according to Lyman et al. (11), and digestibility as described by Kanazawa et al. (12).

RESULTS AND DISCUSSION

The effect of autoclaving and storage on the formation of lipid-protein complexes in the formulated soybean model mixtures can be deduced from the following results

A- AUTOCLAVING OF SOYBEAN MIXTURES

1- Spot test: This test indicated that lipid-protein complexes were formed in all the autoclaved mixtures.

2- Peroxide value: All the oils extracted from the autoclaved soybean mixtures, namely, I Au, II Au, IIIAu, and VAu, showed no change in the peroxide values which gave zero values.

3- Changes in free and bound lipid contents: Bound lipids are taken as a criteria for lipid- protein complex formation.

Changes in the free and bound lipid contents of the mixtures autoclaved at 1.8 atmospheres for 2 hours are given in Figures 3 and 4 .

There is a general increase in the free content accompanied by a general decrease in the bound lipid content of for all the examined mixtures. These results are contrary to what was expected.

Pokorny et al. (13) stated that the weight of lipids could increase by the absorption of oxygen during various oxidation reactions . This might explain the high increase in the free lipid content. The decrease in bound lipid content of most of the samples might be due to the losses of volatile substances during the removal of solvents.

Same authors (13) discussing methods of analysis of bound and free lipids ,reported that methods of analysis should be selected, and that some lipids remain bound and cannot be split.

In this work the decrease in the bound lipid content might have resulted from the fact that water saturated butanol was not the suitable solvent to extract the bound lipids.

4- Spectrophotometric analysis: The absorption spectra of the chloroform extract of the soybean mixture treated by autoclaving IVAu together with a control

sample for comparison are given in Figure 5. The optical densities of the obtained spectra were lower than that of control.

5- Changes in the nitrogen solubility and digestibility of the meal fraction

During food processing the food or protein in the food may be exposed to high temperatures and pressure. Therefore the effect of steam autoclaving at 1.8 atmosphere for 2 hours on the different mixtures was investigated.

Results in figures 6 and 7 reveal a big drop in nitrogen solubility and digestibility of investigated mixtures when compared to the control. The soybean meal has a nitrogen solubility value of 106.5% value and digestibility of 100% while the autoclaved cake has a nitrogen solubility of 22.4% and digestibility of 70.6%.

Nitrogen solubility values for autoclaved mixtures were 21.5, 20.8, 20.2 and 18.1% for I Au, IIAu, IIIAu, and IVAu, respectively.

Digestibility values ranged from 49.3 to 56.5% for I Au and IVAu, respectively.

Altschul (14) reported that lessened solubility is perhaps the most common physical evidence of a protein which has undergone denaturation by heat. The solubility of a protein usually reflect the amount of treatment received during processing.

Under high temperature, pressure, and mixing encounter in extrusion processing, oxidation of unsaturated lipids may occur. Lipid hydroperoxides and their breakdown products may then attack proteins to form covalent bonds, and reduce solubility (15).

B. STORAGE OF SOYBEAN MIXTURES

1-Spot test: This test proved that all samples contained lipid-protein complexes.

2-Peroxide value: The changes in the peroxide values of the oils extracted from the soybean mixtures resulting from storage for 8 weeks at room temperature and freezing for 1 week then storage for 8 weeks at room temperature are represented in Figures 8 - 10.

It can be observed that storage freezing followed by storage of mixture I which is the soybean meal + crude soybean oil resulted in no appreciable change in the PV of the extracted oils during the 8 weeks of storage.

The effect of protein on lipid autoxidation has been observed, and reported even for mixtures containing low amounts of water. Binding of lipid oxidation products in insoluble lipid-protein complexes with the protein phase was also reported by Pokorny et al. (6).

Comparing mixtures Viand III with mixture I there is quite a big increase in the peroxide values, this is normal due to the presence of oxidized and degummed oxidized soybean oil in mixtures II and III, respectively. Mixture I contains crude soybean oil. This decrease in PV is usually followed by a decrease in PV with increasing time of storage at room temperature or by freezing then storage.

Results of freezing prior to storing, when compared to results of storage at room temperature reveals that freezing before storage caused less formation of lipid-protein complexes, as indicated by the lower peroxide values. Pokorny (16) recommended cold or frozen storage among other conditions for the inhibition of lipid-protein complex formation.

The peroxide value of mixture II is usually lower than III probably due to the antioxidant effect of the phospholipids in mixture II.

3- Changes in the free and bound lipid contents:

Figures 11-13, show the changes in the content of the free as well as the bound lipid in case of soybean mixtures during storage for a period of 8 weeks at room temperature, and the mixtures frozen for one week followed by storage at room temperature for 8 weeks.

The data reveal the decrease in the free lipid content of all stored mixtures with increasing time of storage, this applies to stored mixtures as well as frozen then stored mixtures. The bound lipid content of all mixtures decreases after one week storage then start increasing slightly until the eighth week.

4- Spectrophotometric analysis:

Figures 14a and 14b represent the spectrophotometric analysis of the chloroform extracts of the soybean mixtures, during storage in addition to a control sample (untreated soybean gums). Absorption spectra show decrease in the optical densities by increasing time of storage from one to eight weeks for both storage at room temperature and freezing then storing.

Results in general show that all treatments resulted in absorption spectra of the extracted gums with lower optical densities than that of the untreated gums (control), which might be due to a complex formed between phospholipids and protein.

Pokorny et al. (6), reported that the binding of oxidized phospholipids with protein is evident. They found that the weight of extracts from casein-phospholipid mixtures decreased by storage because of the transformation of phospholipids into insoluble complex compounds.

5-Nitrogen solubility and digestibility of the meal fraction :

The effect of storage for 1, 3, 5, and 8 weeks on the nitrogen solubility and digestibility of the meal protein resulting from mixtures under investigation are represented in Figures 15 and 16. It can be seen that the effect of storage time on both nitrogen solubility and digestibility is so little that it is not worth discussing.

Figures 17 and 18 show the effect of freezing for one week then storage for eight weeks on the nitrogen solubility and digestibility of the meal protein resulting from the treated soybean mixtures.

Comparing results of freezing then storage with storage alone, it is clear that freezing for one week before storage causes less decrease in the digestibility and nitrogen solubility of the same samples that were stored without freezing. Pokorny et al. (17),reported that products of oxidized lipids with protein resulted in decrease in the digestibility of both the lipid and protein fractions due to incomplete and slower enzymatic hydrolysis of bound lipids and proteins.

CONCLUSION

Results of this study can be concluded as follows:

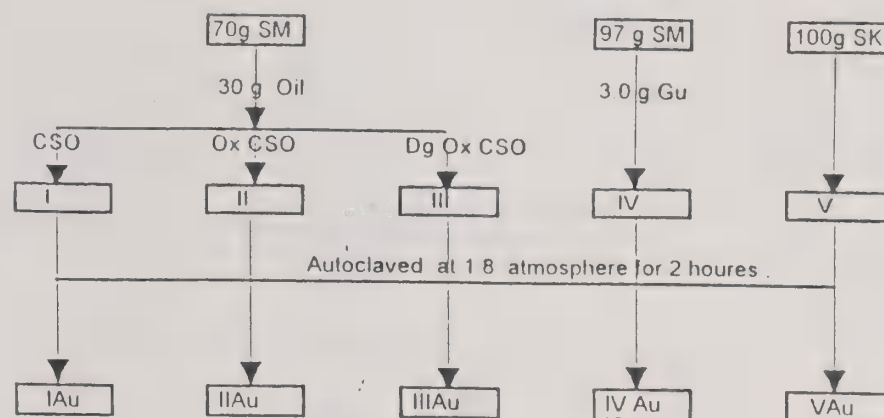
Autoclaving caused no change in the peroxide value ,free and bound lipids of the oils extracted from the investigated mixtures.The decrease in the optical densityof the gums indicated the formation of phospholipid-protein complex. The nitrogen solubility and digestibility of the meal protein were highly damaged probably due to denaturatio which is known to enhance lipid-protein complex formation.

Storage of the mixtures with crude oil resulted in no change in the PV, while storage of mixtures with oxidized oils and degummed oxidized resulted in an increase in PV. Degummed oxidized oils gave lower PV than oxidized oils with gums ,due to the antioxidant effect of the phospholipids. This indicates the formation of lipid-protein complexes. Absorption spectra indicated the possibility of phospholipid-protein complexation. Nitrogen solubility and digestibility of the meal protein was not affected by storage.

Free and bound lipid contents could not be taken as a relevant criteria for lipid-protein complex formation.

REFERENCES

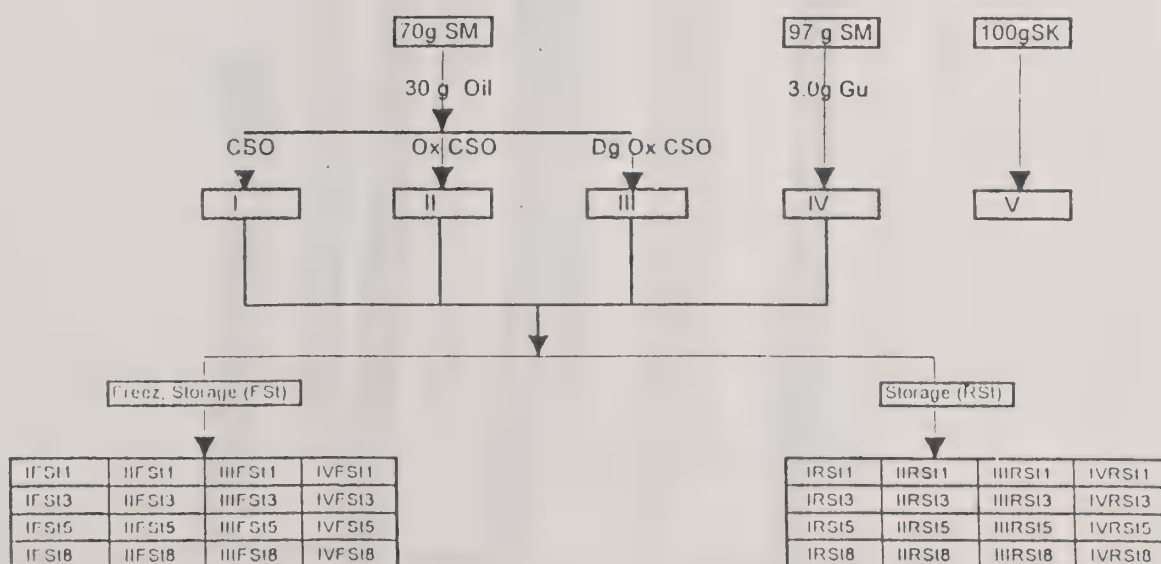
- 1-Hoseney R.C., Y.Pomeranz and K.F. Finney, Cereal Chem. 66: 47, 1989.
- 2-Karel M., J. Food Science 38 :756, 1973.
- 3-Wu L.C. and Bates R.P., J. Food Science 40 : 160, 1975.
- 4-Chio K. and A. Tappel, Biochem. 8: 2821, 1969.
- 5-Chapman D., lipids 4: 751, 1969.
- 6-Pokorny J., S.Smidrkalov, H.Zwain and G.Janicek, Nahrung 19: 635, 1975.
- 7-Cousins E.R., S.P.Fore, H.J. Janseen, and R.O.Feuge, JAOCS 30: 9, 1953.
- 8-BekesF., U.Zawistowsks and W. Bushuk, Cereal Chem 60: 371, 1983.
- 9-A.O.A.C. Official Methods of Analysis. Washington D.C., 13th Edition 1980.
- 10-Clark E.P., ' Semimicro Quantitative Organic Analysis', Academic P ress,N.Y. 1943.
- 11-LymanC.M., W.Y. Chang and J.R.Couch, J. Nutrition 49: 679, 1953.
- 12-Kanazawa K., H. Ashida and M.Natake, J. Food Science 52 : 475, 1989.
- 13-Pokorny J., G. Janicek and J.Davideh, Zeszyty Problemowe Postepow Nauk Rolniczych , 167: 155, 1975.
- 14-Altschul A.M. , 'processed plant protein foodstuffs', Academic PressInc.N.Y., 1983.
- 15-Izzo M. and C.T.Ho, Cereal Chem. 66 : 47,1989.
- 16-Pokorny J.,La Rivista, Italiana Delle Sostanze Grasse LIV Settembre, 389, 1977.
- 17 -Pokorny J.,E.N.Moravkova and H.Alexova, Proceedings of the 16th IFS Congress, 603, Budapest, 1983.



Examples :

III Au : Model mixture III treated by the autoclave .

Figure (1) : Schematic Representation of the Treated Soybean Mixtures after Autoclaving .



Examples

IIRS15 : Model mixture II treated storage at room temperature for 5 weeks

IIIFS13 : Model mixture III treated by freezing then storage for 3 weeks

IVRS18 : Model mixture IV treated storage at room temperature for 8 weeks

Figure (2) : Schematic Representation of the Treated Soybean Mixtures During Storage .

Figure (3) : Changes In the Free Lipid Content of Treated Soybean Meal Mixtures after Autoclaving .

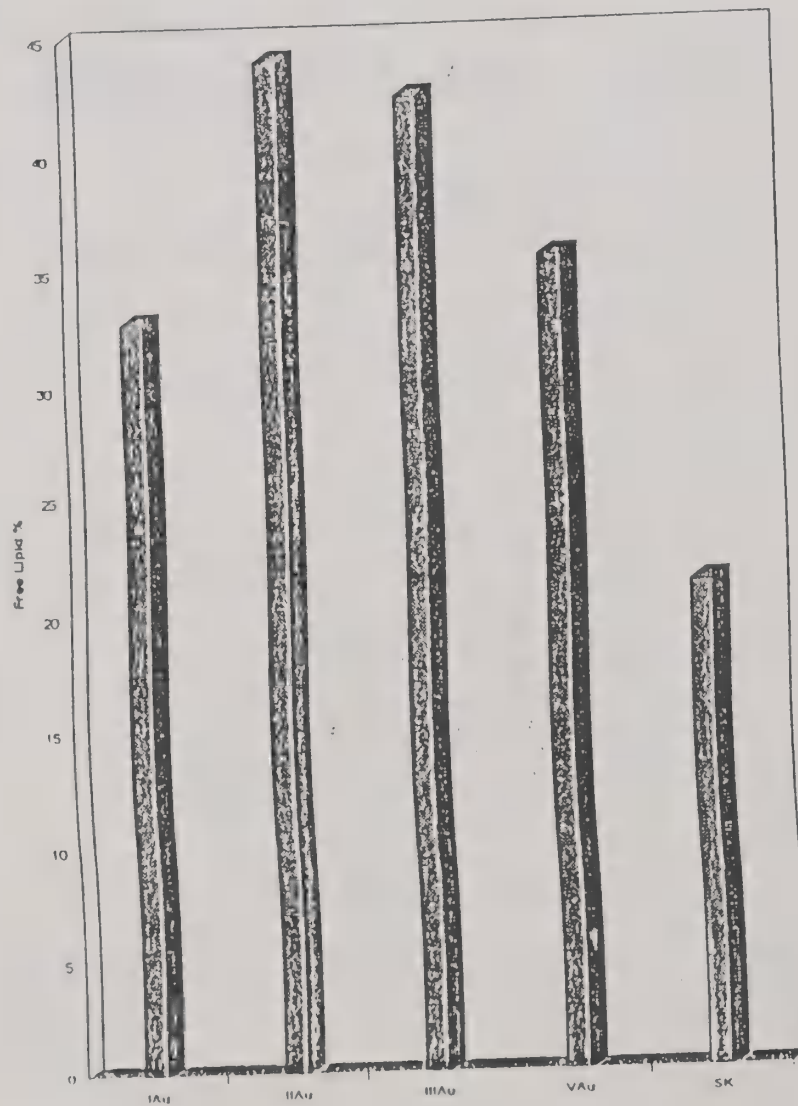
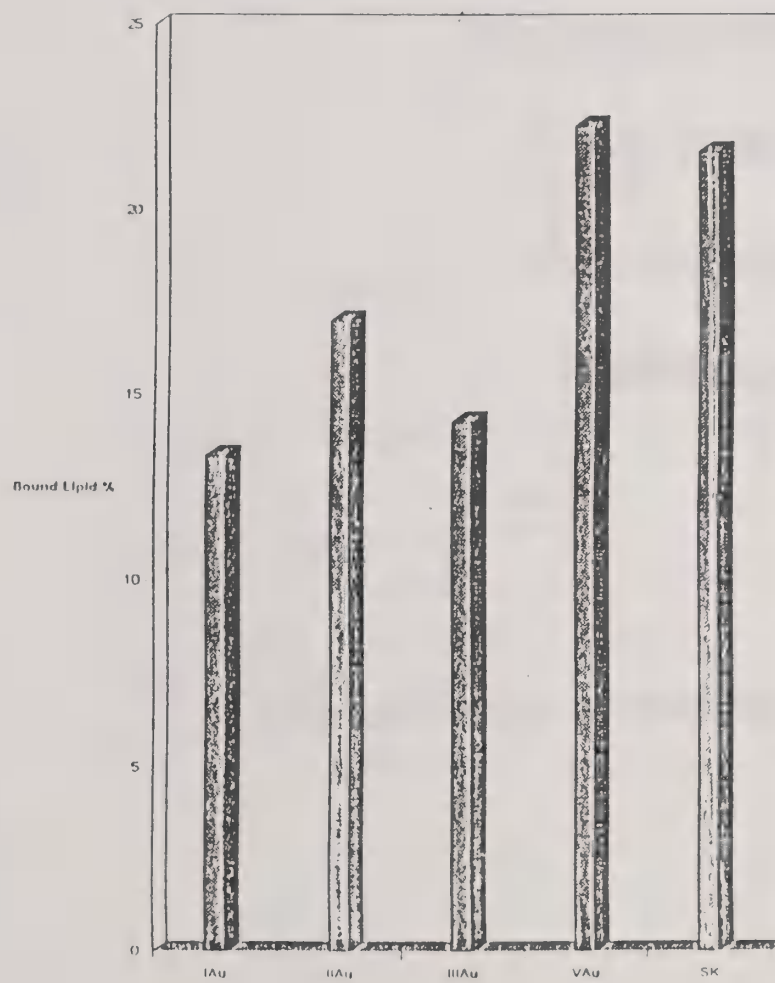


Figure (4) : Changes in the Bound Lipid Content of Treated Soybean Meal Mixtures after Autoclaving .



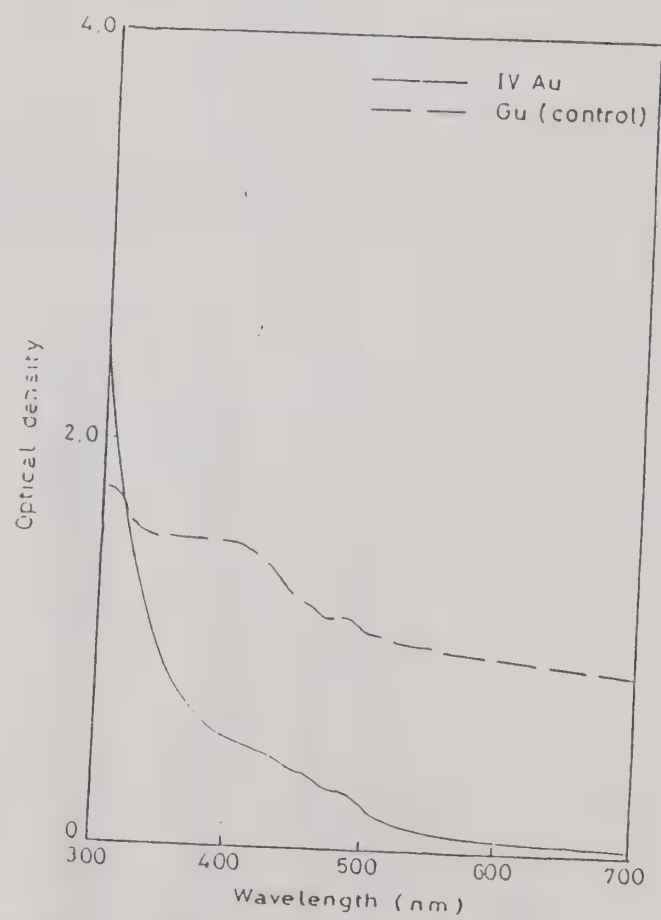


Fig. (5) : Absorption Spectra of Gums Extracts from Soybean Mixtures after Autoclaving and Untreated Gums (Gu).

Figure (7) : Changes in the Digestibility of Treated Soybean Meal Samples after Autoclaving .

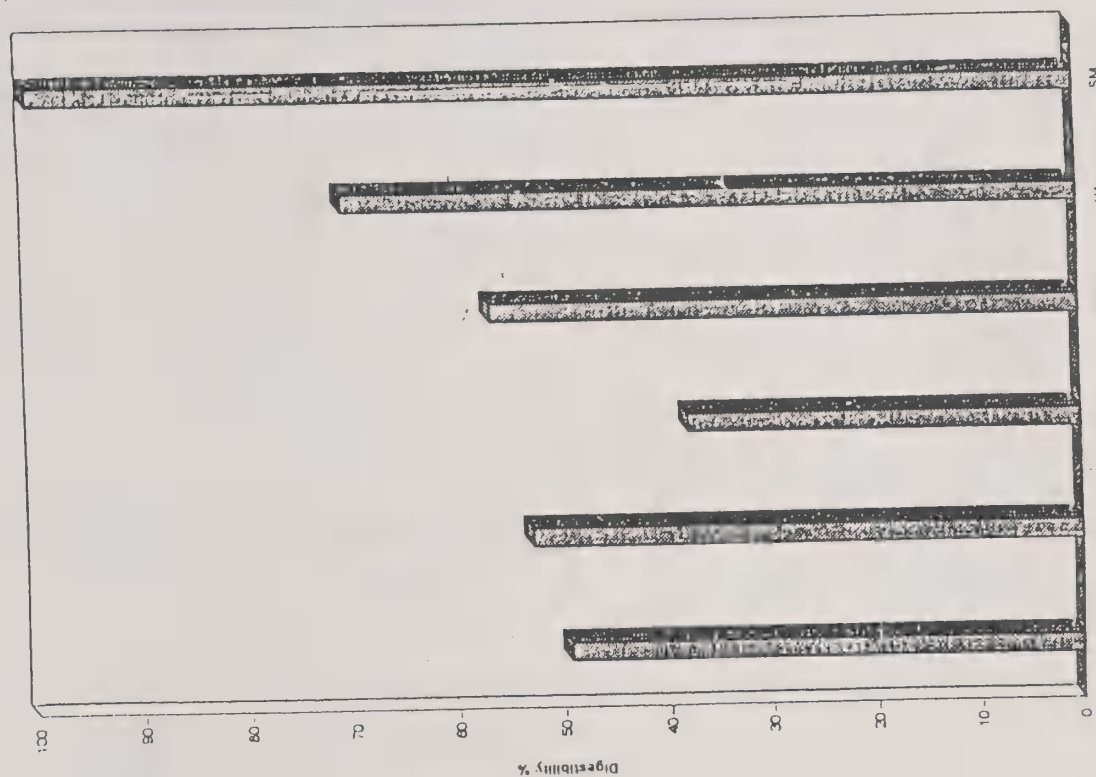


Figure (8) : Changes in the Nitrogen Solubility of Treated Soybean Meal Samples after Autoclaving .

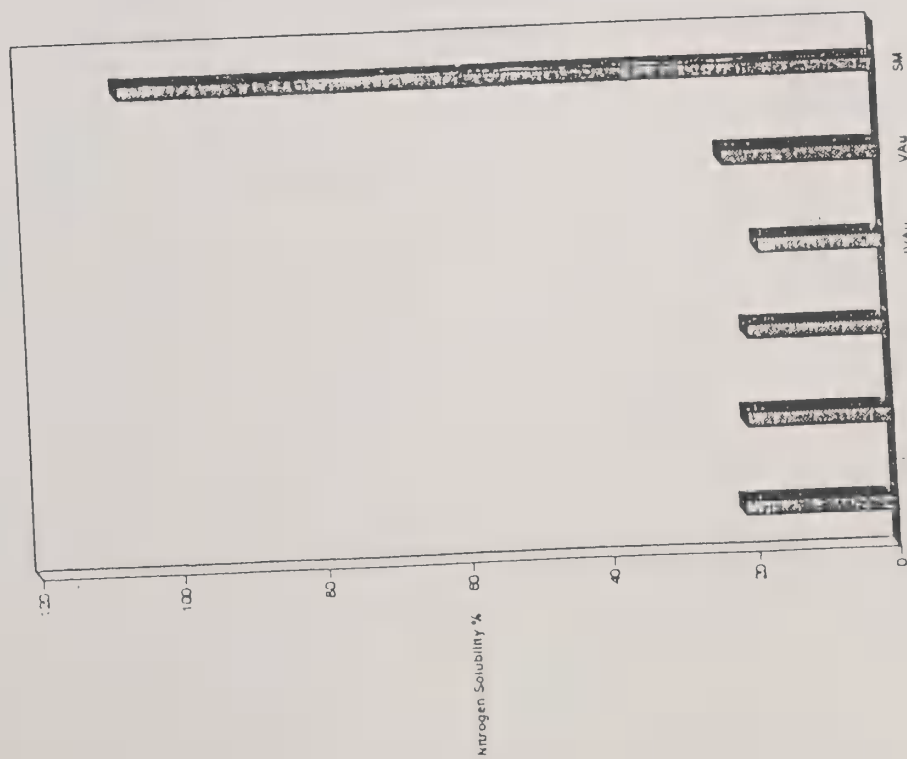


Figure (8): Changes in the Peroxide Value of the Extracted Soybean Oil Samples from Soybean Mixtures During Storage .

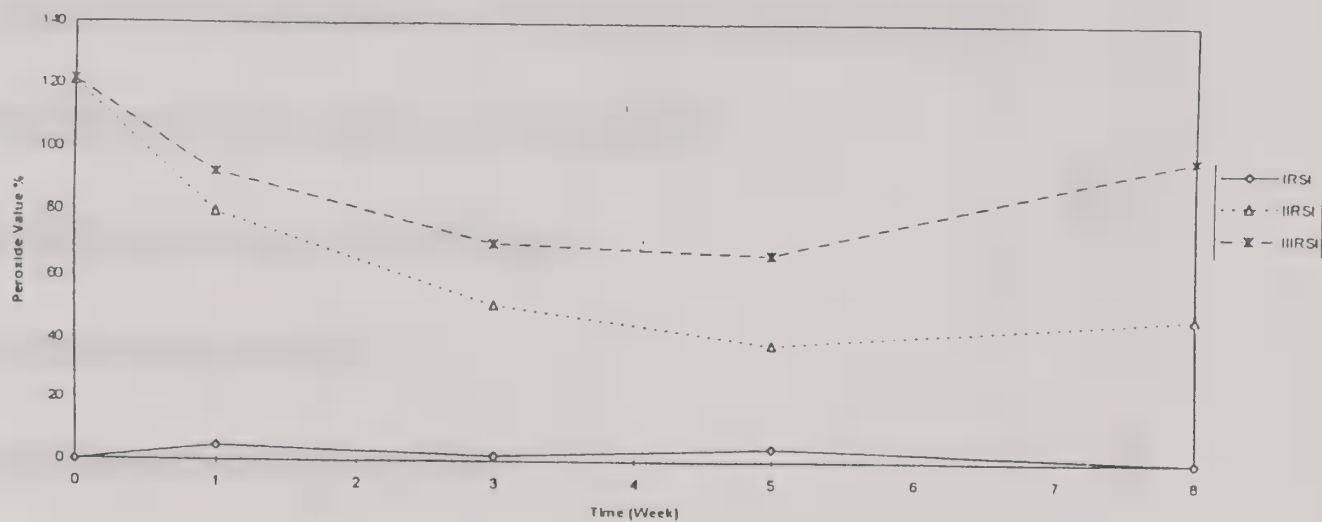


Figure (9): Changes in the Peroxide Value of the Extracted Soybean Oil Samples from Soybean Mixtures During Freezing then Storage .

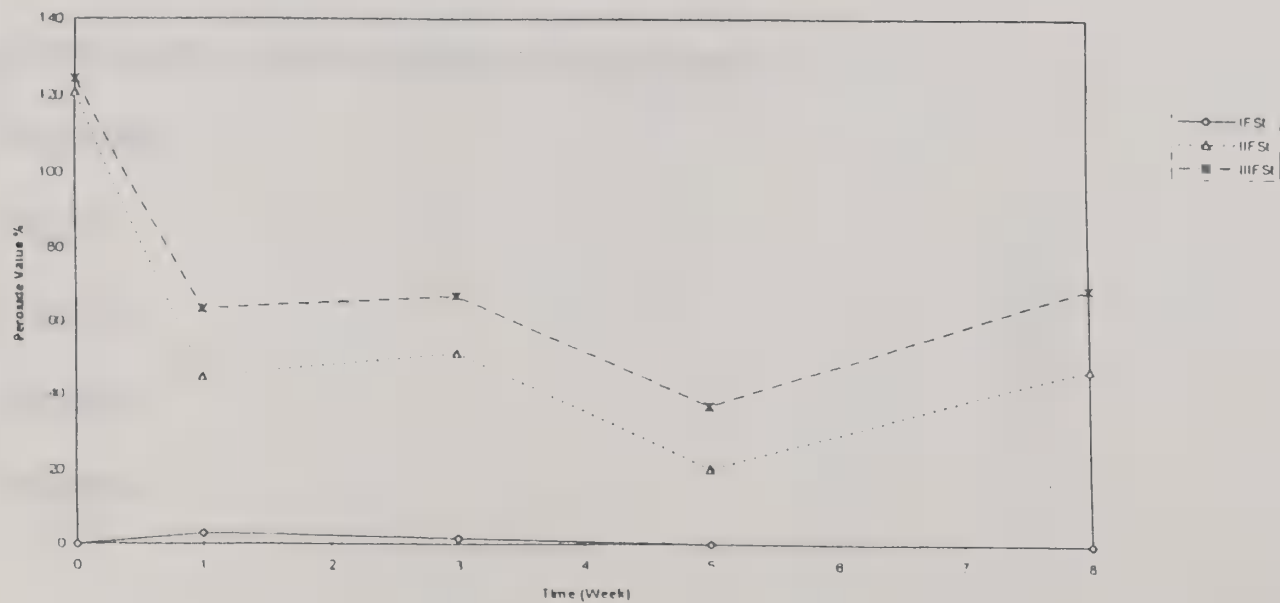


Figure (I) : Changes in the Free Lipid Content of Treated Soybean Meal Mixtures During Storage .

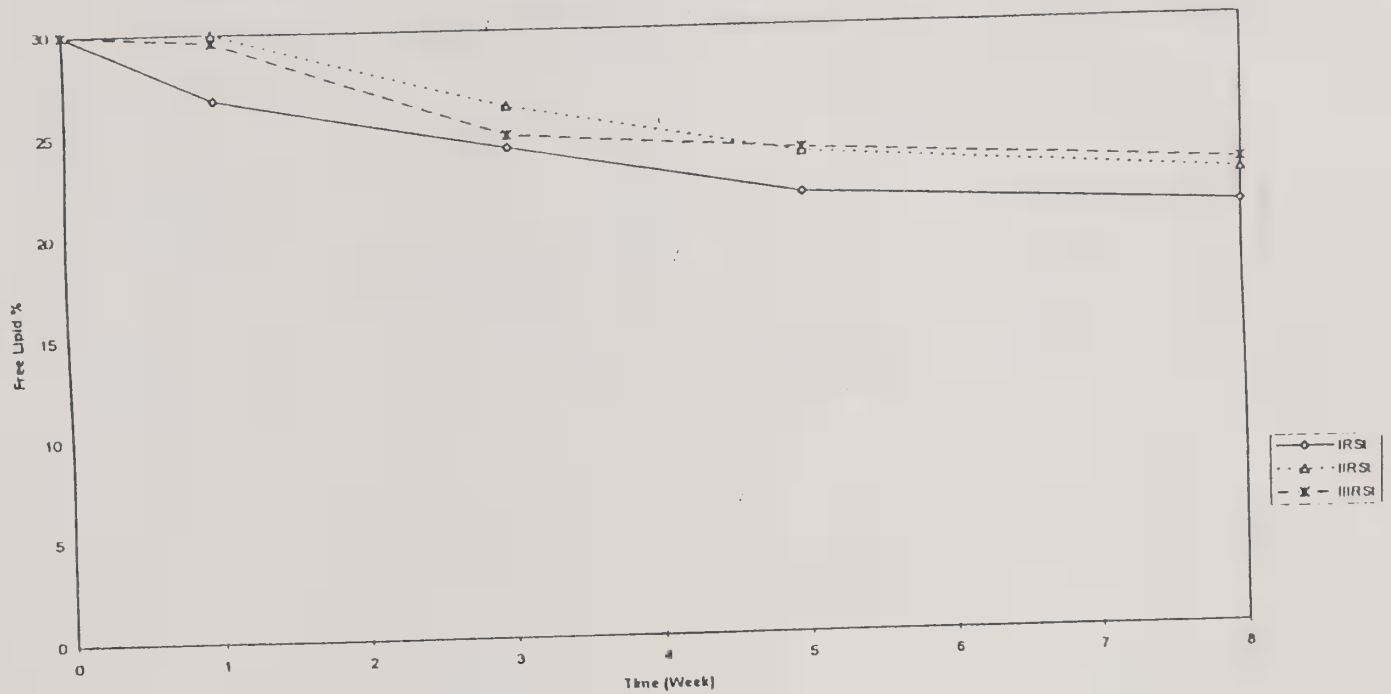


Figure (II) : Changes in the Bound Lipid Content of Treated Soybean Meal Mixtures During Storage .

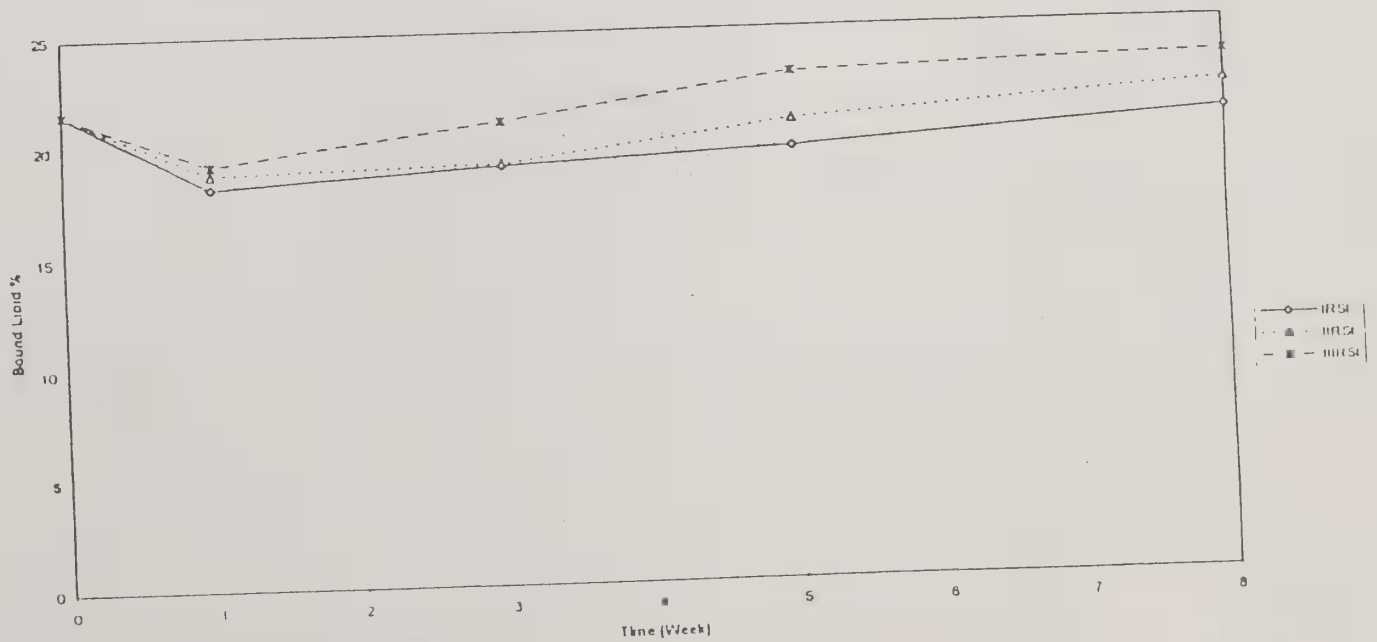


Figure (12) : Changes in the Free Lipid Content of Treated Soybean Meal Mixtures During Freezing then Storage .

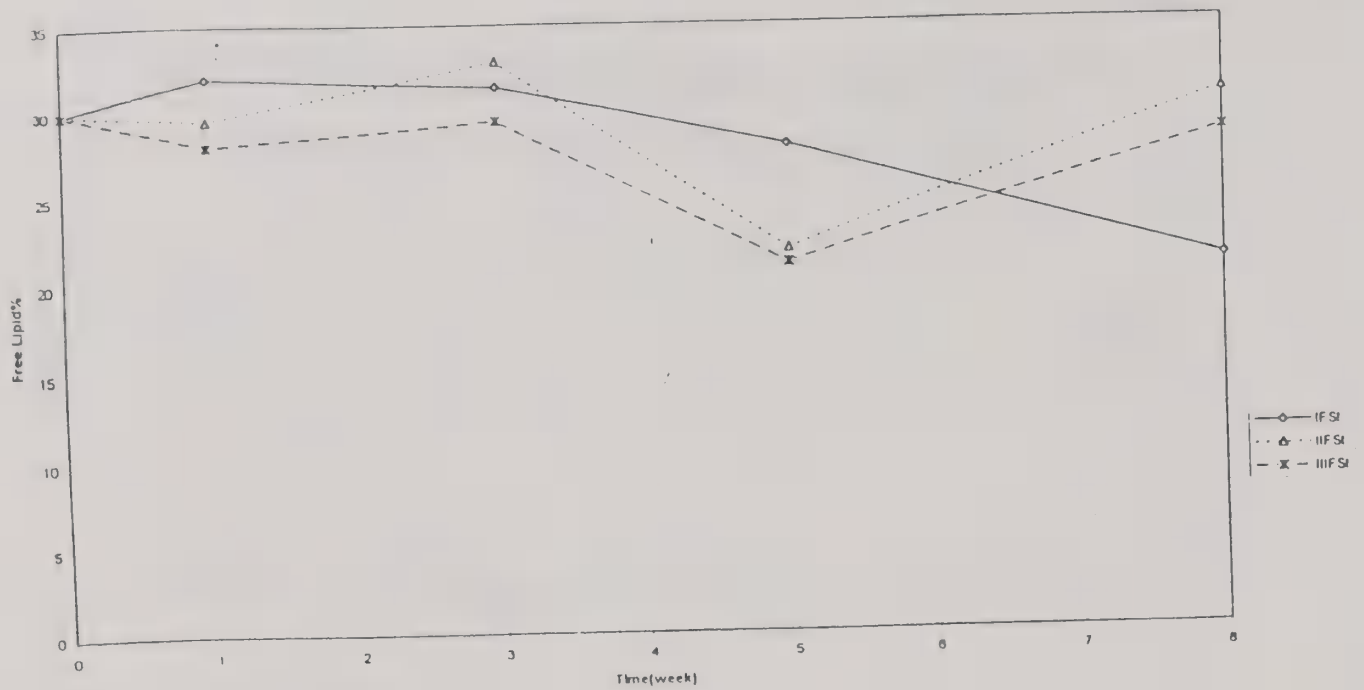
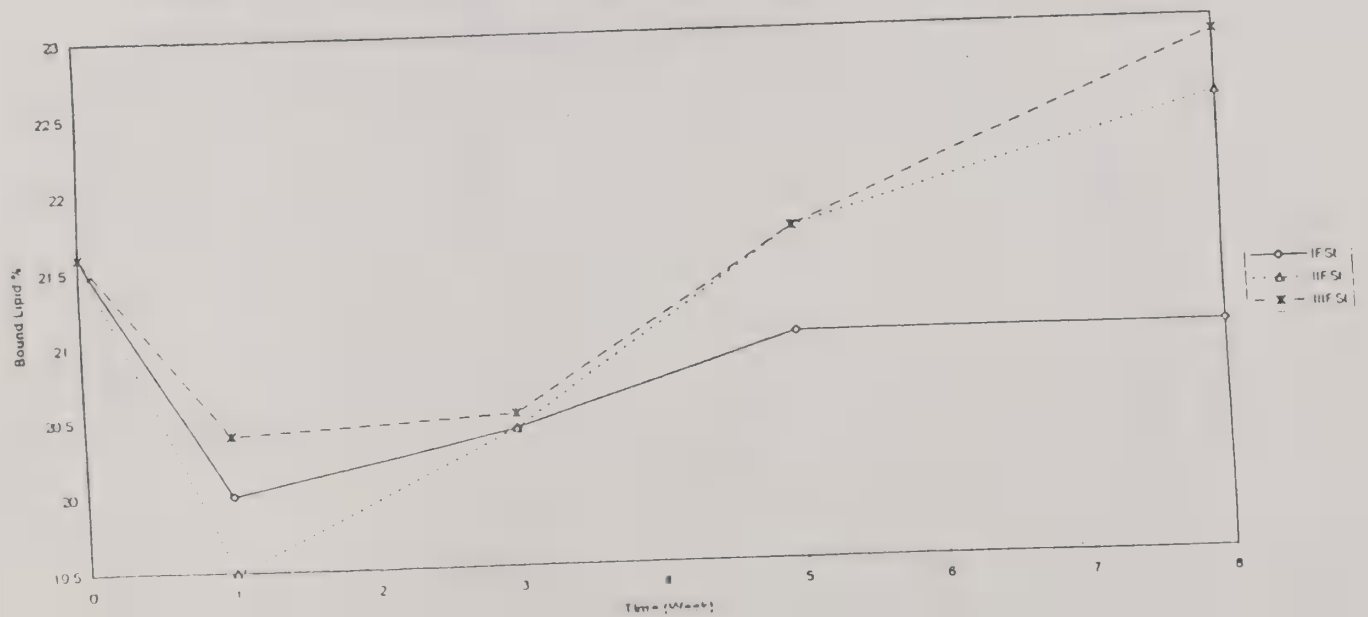


Figure (13) : Changes in the Bound Lipid Content of Treated Soybean Meal Mixtures During Freezing then Storage .



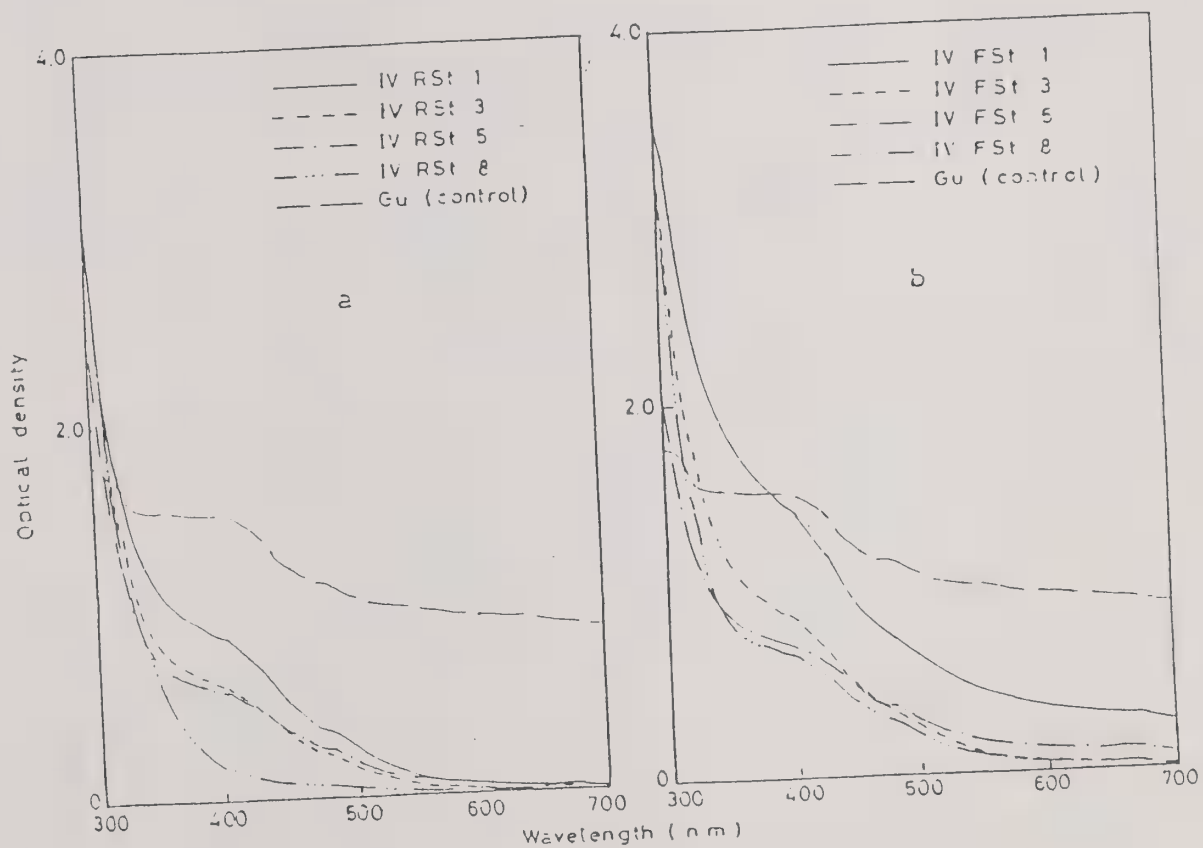
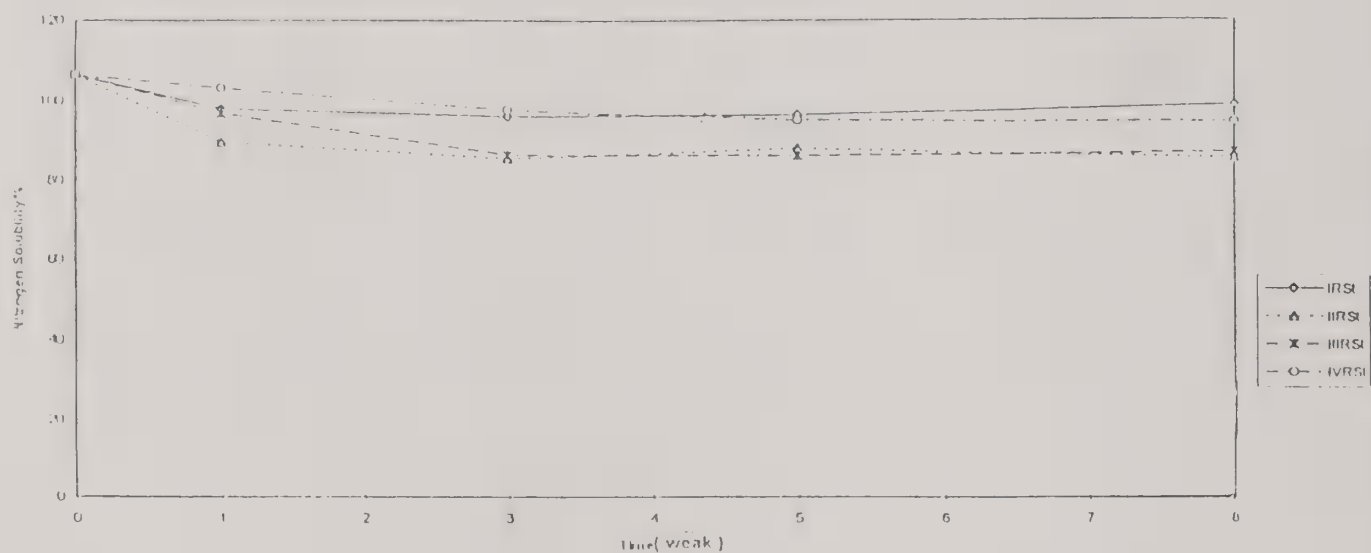
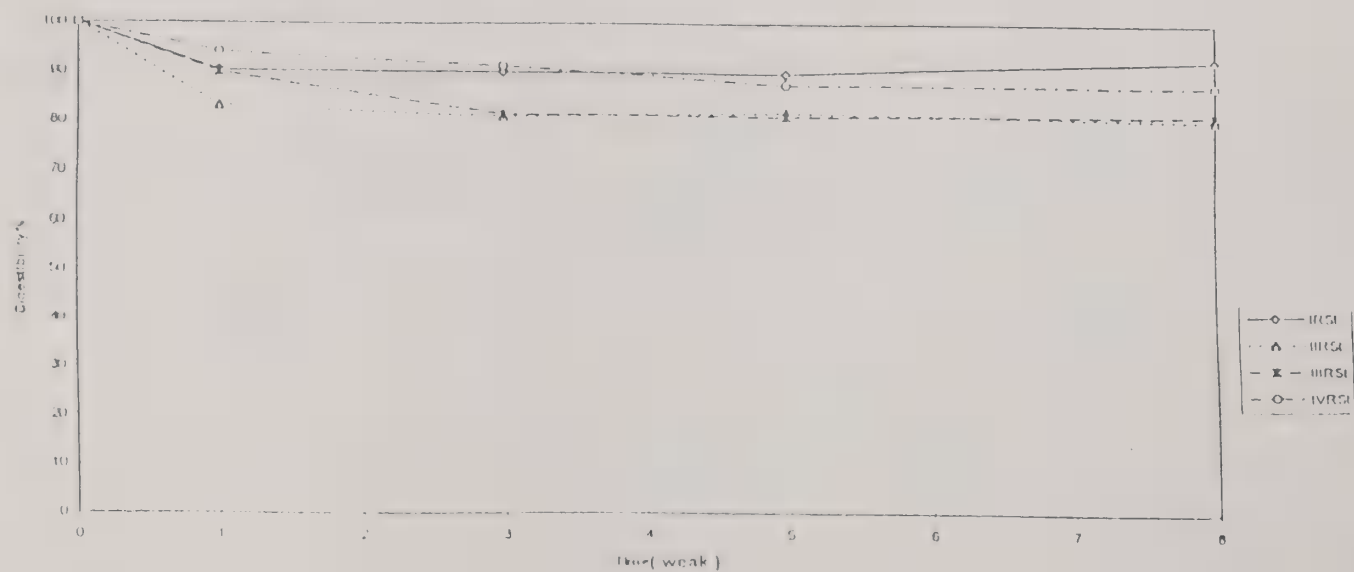


Fig. (14): Absorption Spectra of Gum's Extracts from Soybean Mixtures During Storage (a. IV RSt and b. IV FSt) and Untreated Gums (Gu).

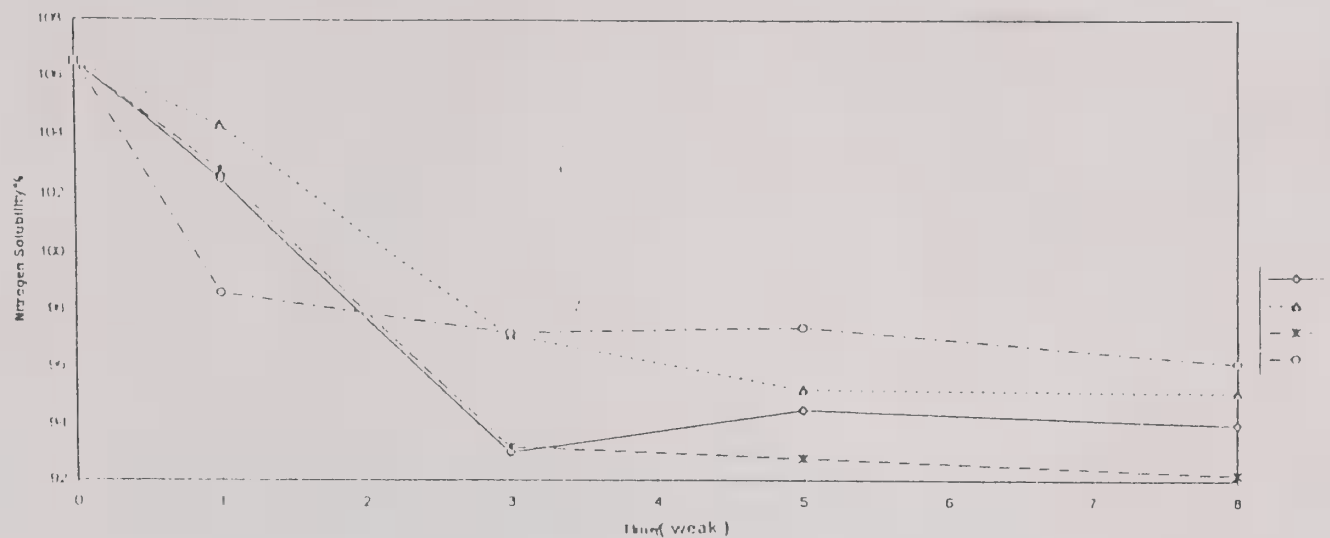
Figure(7) : Changes in the Nitrogen Solubility of Treated Soybean Meal Samples During Storage .



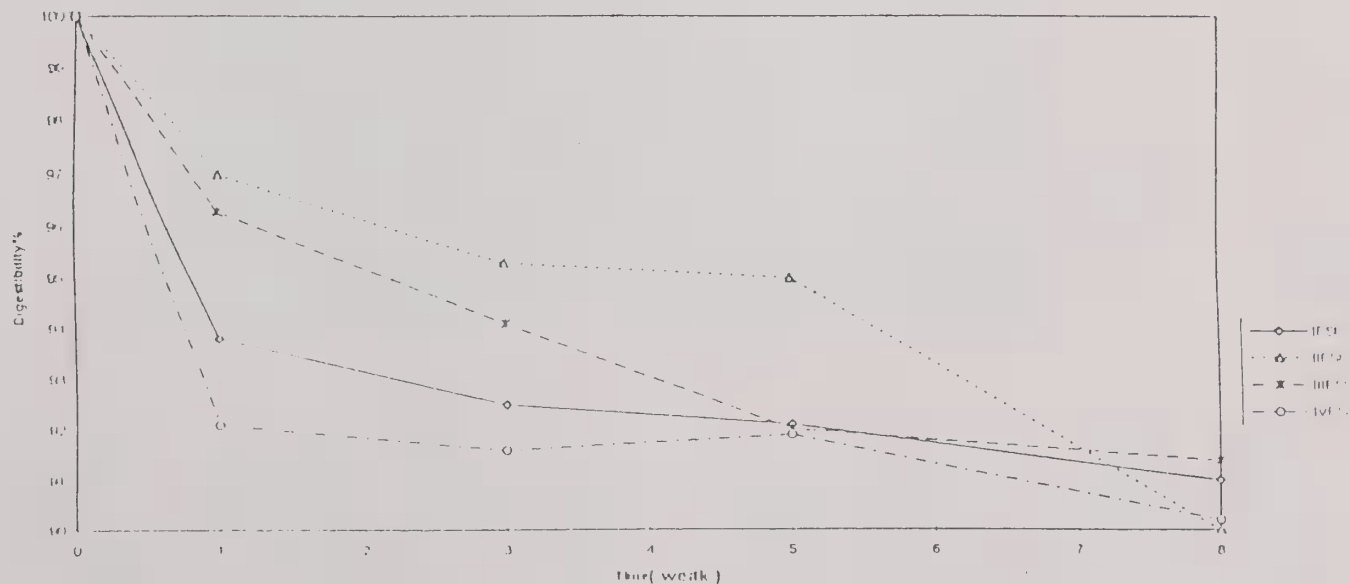
Figure(8) : Changes in the Digestibility of Treated Soybean Meal Samples During Storage .



Figure(17) : Changes in the Nitrogen Solubility of Treated Soybean Meal Samples During Freezing then Storage .



Figure(18) : Changes in the Digestibility of Treated Soybean Meal Samples During Freezing then Storage .



*Effect of Lipid-Protein Complexes on the
Protein Fraction of Soybean*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Effect Of lipid-Protein Complexes On The Protein Fraction Of Soybean

F. S. Taha, E. A. Abd El-Motaal, H.E. Helmy, and Z.E. Shoeb

Fats and Oils Department, National Research Centre, Dokki, Cairo, Egypt.

INTRODUCTION

Many natural and processed foods contain lipid-protein complexes in which nonpolar and polar lipids are dispersed and often held by proteinaceous networks.

Changes in proteins are often affected by many constituents of the food eg. minerals, acids, vitamins and lipids. All types of lipids especially polar lipids, form lipid-protein complexes with protein on storage. These lipid-protein complexes can be easily split again into its lipidic and protein constituents. Polar groups of oxidized lipids form non-extractable compounds with protein more readily than polar groups of monoglycerides (1). The formation of non extractable compounds is due both to the interaction of protein with hydroperoxides, and with non-peroxidic oxidation products. Products of oxidized lipids with protein results in decrease in the digestibility of both the lipid and protein fractions due to incomplete and slower enzymatic hydrolysis of bound lipids and proteins (2).

Denaturation causes increased lipid oxidation (3), native soybean globulins do not form lipid-protein complexes, prior dissociation with denaturing agents is needed (4).

Lipid-protein complexes have specific properties and generally a lower nutritional value due to a decrease of biological value of protein, and changes in the digestibility. The formation of these complexes can also affect the sensoric properties of foods.

The aim of the present study is to investigate the effect of the presence of lipid-protein complexes on the isoelectric precipitation curve, electrophoretic pattern, and some functional properties of the soybean meal protein.

MATERIALS AND METHODS

Materials. materials used in this investigation include:

1- Oxidized soybean oil: soybean oil supplied by El-Badrachin Factory, Giza, Egypt, was heated in a flat petri dish in an oven at 100 °C for a period of three days, then the oil was left at room temperature for six days until the peroxide value of the oil reached a range between 119-126.

2-Soybean meal: soybean meal from El-Badrachin Factory was re-extracted using commercial hexane until it reached a value of less than 1% residual oil.
3-Soybean cake: This was ground soybeans containing both the oil and protein, also supplied by El Badrachin Factory.

Mixtures from the above mentioned materials exposed to conditions that proved from our previous studies (5, 6) to enhance the formation of lipid-protein complexes, were now investigated with the aim of determining the effect of lipid-protein complexes on the isoelectric precipitation curve, electrophoretic pattern, and some functional properties of the protein of the mixtures.

These mixtures include:

Mixture I.: Soybean meal + oxidized soybean oil heated for 2 days at 95°C.

Mixture II.: Soybean meal + oxidized soybean oil + 50% moisture, heated for 1 day at 100°C.

Mixture III.: Soybean meal + oxidized soybean oil autoclaved at 1.8 atmosphere for 2 hours.

Mixture IV.: Soybean meal + oxidized soybean oil stored at room temperature for three weeks.

Mixture V.: Soybean cake heated at 95°C for 2 days.

Mixture VI.: Soybean cake + 50% moisture heated for 1 day at 100°C.

Mixture VII.: Soybean cake autoclaved at 1.8 atmosphere for 2 hour

SM.: Defatted soybean meal (control).

In mixtures I- IV, 70g soybean meal were mixed with 30g oxidized soybean oil and subjected to the above mentioned conditions.

The seven mixtures were then defatted with n-hexane and the meals subjected to 1- Determination of the isoelectric precipitation curves of the protein, 2- Determination of the electrophoretic pattern of the protein, 3- Determination of some functional properties of the protein.

Methods: The isoelectric precipitation curve was carried according to El-Nockrashy et al., (7) and (Taha et al., (8). The electrophoretic pattern of the protein was carried using sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) according to the method of Leammli (9). The functional properties were determined according to standard procedures . Nitrogen solubility index NSI (10), oil holding capacity (11), water absorption capacity (12), gelation (13), and emulsifying capacity (14).

RESULTS AND DISCUSSION

Mixtures containing oxidized soybean oil were chosen because oxidized oil was reported to be essential for lipid-protein formation (15-17) . Our previous results

(5, 6) also showed decrease in solubility and digestibility of the protein of these mixtures indicating the propability of lipid-protein complex formation .

1. Effect of Lipid-protein Complexes on the Isoelectric Precipitation Curves of the Protein.

Figure 1. represents the isoelectric precipitation curves of the meal protein resulting from mixture I: soybean meal + oxidized soybean oil heated for 2 days at 95 °C ; mixture II : soybean meal + oxidized soybean oil + 50% moisture, heated for 1 day at 100° C; and SM : defatted soybean meal, acting as control.

Figure 2. represents the isoelectric precipitation curves of the meal protein resulting from mixture III: soybean meal + oxidized soybean oil autoclaved for 2 hours at 1.8 atmosphere ; mixtureIV : soybean meal + oxidized soybean oil ,stored for 3 weeks at room temperature ; and defatted soybean meal (control).

Comparing the isoelectric precipitation curve of the defatted soybean meal to the isoelectric precipitation curves of the soybean meal protein resulting from the different mixtures containing different quantities of lipid -protein complexes

(figures1 and 2), it can be clearly observed that the presence of lipid-protein complexes causes some changes in the precipitation curves , which can be summarized as follows:

The isoelectric point (IEP) of the defatted soybean meal is at pH 4.6 and remained unchanged in all samples except the moist heated sample (mixture II),which showed a shift in the IEP to pH 4.2 .

The amount of protein precipitated at the IEP differed for each meal sample. Protein precipitated was 86.8% , 76.5% , 53.3% , 47.1% , and 83.5% , for defatted soybean meal, and the meals resulting from mixtures I , II , III , and IV , respectively.

Highest % precipitated protein lies between pH 4.0-5.0 for the defatted soybean meal and the meals of mixtures I and IV, . For the moist heated mixture II, and autoclaved mixture III, highest % protein precipitated lies between pH 3.0 - 5.0, and 3.0-4.8, respectively.

Minimum precipitation or maximum solubility of the protein for defatted soybean meal, and meals from mixtures I, II, IV, and III, was found from the precipitation curves to be at pH 9.0, 8-10, 6.0, 9.0 and 8.0, respectively, where 96.7%, protein was solubilized for the soybean meal as well as meals from mixtures I, II, IV, and 99.9% solubilized protein for meal of mixture III.

The precipitation curve of the meal protein resulting from the moist heated mixture II, differed the most from the precipitation curve of the defatted soybean meal, while that of the meal protein resulting from the stored mixture IV is the closest to that of the defatted soybean meal. Results of solubility and digestibility for protein of stored mixture (6) confirm this finding, as they both show least decrease when compared to the defatted soybean meal, indicating least formation of lipid-protein complexes. Whereas both the solubility and digestibility of the meal protein resulting from the moist heated mixture was the most affected also the peroxide value of the oil resulting from this mixture was also highly affected (5).

The meal resulting from the autoclaved mixture III also differed in its precipitation curve, when compared to defatted soybean meal, % precipitation was much less than for the defatted soybean meal.

Wolf (18) reported the isoelectric region of the main protein of soybean to be between pH 4.6-5.0.

This change in the isoelectric precipitation curve of the meal protein resulting from the different mixtures is probably due to change in the protein structure, caused by the presence of lipid-protein complexes. This change might affect some processing steps like during the preparation of protein isolates.

2. Effect of Lipid-protein Complexes on the Electrophoretic Pattern of the Protein.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was proven to be a valuable tool for showing the molecular weight of proteins. In this study it was also used to show changes in molecular weight of the proteins, that might happen due to lipid-protein complex formation.

Figure 3. represents the SDS-PAGE gel patterns for the tris buffer extracts of seven meal proteins resulting after defatting of the following mixtures: I, II, III, IV, V, VI, VII, as well as the defatted soybean meal (SM) for comparison. The mixtures I-IV were chosen as previously explained, while mixtures containing the cake V-VII were chosen because the cake represents the oil and protein fractions as they exist naturally in the soybeans, these mixtures were subjected to the same conditions as the formulated mixtures I-III.

The gel contained nine lanes (figure3.).The first lane represents the molecular weight markers (MW: 66, 45, and 29 K Dalton),then seven lanes that represent the meal protein resulting from the mixtures I-VII , as well as the lane representing the defatted soybean meal, as control.

Figure 4 is the plot between the log. molecular weight of the markers and their relative electrophoretic mobility. This acts as a standard curve for protein predicting

molecular weights, of various bands of the investigated protein samples.

The SDS-PAGE gel patterns reveal that:

For the defatted soybean meal (control ,lane g), there are three subunits.

- Subunit A with the highest molecular weights above 35 K Da.
- Subunit B with the lowest molecular weights below 10.45 K Da.
- Subunit C with molecular weights between subunits A and B i.e. between 35-10.45 kDa.

Subunit A is composed of 12 major bands with molecular weights: 75, 70,62, 66, 58, 46.5, 45, 41.5, and 35 K Da

Subunit B is composed of 6 bands that are identified as soybean trypsin inhibitor.

Subunit C is composed of 7 bands with molecular weights 30, 29, 26.5, 23.5, 22,20 and 10.8 K Da.

The bands for the protein extracted from mixtures I (lane b) and mixture II(lane i),all disappeared except two very faint bands with molecular weights 35 and 10.45 K Da.that still appeared. This means that dry heating for two days or moist heating for 1 day with 50% moisture accelerate the interaction between the oxidized lipids and proteins to form complex compounds that cause the change in the electrophoretic pattern when compared to the control. The two protein samples resulting from autoclaving of mixtures III (lane d),and VII (lane e), also show disappearance of most of the protein bands. Only two very faint bands with molecular weights 35 and 10.45 K Da appear. This indicates that autoclaving for 2 hours, probably causes denaturation or formation of lipid-protein complex, thus causing the change in the electrophoretic pattern.

Mixture IV resulting after storing at room temperature (lane h) shows an electrophoretic pattern similar to that of the defatted soybean meal (control), the only difference occurred to the bands with molecular weights above 70 K Da , these bands disappeared.

The dry heated soybean cake , mixture V (lane c) shows a similar electrophoretic pattern to the control (lane g) .

The moist heated soybean cake, mixture VI (lane f)results in disappearance of the high molecular weight bands above 42.5 K Da. The rest of the electrophoretic pattern was the same as that of the control (lane g).

The disappearance of protein bands from the different treated soybean mixtures might be due to the formation of a complex resulting from the interaction of the oxidized lipids with the free amino groups of the protein molecule as indicated by protein resulting from dry heated mixture I, moist heated mixture II, and autoclaved mixture III. These mixtures all contain oxidized soybean oil. These results are in agreement with previous results of solubility and digestibility (5,6). The electrophoretic pattern of the dry heated cake ,containing no oxidized oil,was similar to that of control. Moist heating of cake showed some change in the electrophoretic pattern.Because autoclavingcauses denaturation, which is reported to be necessary for lipid - protein complex formation , The results showed that the autoclaved cake is the most affected sample of the cake samples.

3. The Effect of lipid-protein Complexes on Some Functional Properties of Soybean Meal Protein.

The functional characteristics of protein in protein containing products play a bigger role than nutritional consideration in determining their acceptability as ingredients in prepared foods. Finch(19) , stated that protein may be used in foods for their functional properties as well as their nutritive properties.

Physicochemical properties of oilseed flours depend upon naturally occurring characteristics associated with the kernels as well as processing conditions to which the kernels were exposed during oil extraction and conversion to flour. Thus the interrelationship of protein quality and processing conditions, where interactions between proteins, lipids , carotenoids , chlorophyll, and other ingredients are possible, influence the functional performance of oilseed protein in food systems. Many factors influence the functional properties of protein including, moisture , temperature, pH, concentration , reaction time, enzymes , chemical additives, and mechanical processing .

The functional properties investigated were: 1- Nitrogen solubility index; 2- Water absorption capacity;3-Oil holding capacity; 4- Gelation; 5- Emulsifying capacity.

Table 1. gives the results of the investigated functional properties of the meals resulting from the seven chosen mixtures,as well as the defatted soybean meal as a control.

Nitrogen Solubility Index (NSI).

Nitrogen solubility index is a very important measure of the functionality of the protein in many food systems .

NSI of the protein resulting from the soybean mixtures containing the oxidized soybean oil ,as expected and in agreement with previous results (5,6)

are very much damaged. Results show % decrease in NSI of 84%, 86%, 87%, and 73% for mixtures I, II, III, IV, respectively.

Also that storage results cause less protein damage than dry heating, moist heating

and autoclaving is also confirmed by previous results (5, 6). Meal samples resulting from the cake mixtures showed 29%, 84%, 86% decrease in NSI over the defatted soybean meal (control) ,for meal of mixtures V, VI, and VII, respectively, confirming results obtained by electrophoresis.

These protein products cannot be used in food systems requiring high protein solubility ,such as :beverages , instant foods, baby foods and others.

Water Absorption Capacity (WAC)

This is defined as the ability of a product to absorb water and swell. This criteria is very important in the manufacture of bakery products, pasta products and doughnuts.

Table 1. shows that all the investigated meal samples have lower WAC than the defatted soybean meal (control). The WAC decreased from 260.0% for the control to 180.0, 200.0, 170.0, 230.0, 220.0, 190.0 %, for meals resulting from mixtures I, II, III, IV, V, VI, VII, respectively. These results indicate that these proteins would not perform well in dough handling systems.

Oil Holding Capacity (OHC)

The oil holding capacity is a measure of the protein ability to bind with oil, and is important in the doughnut and sausage industry (11). Also in meat systems the ability of a protein to absorb oil is important in enhancing its flavor characteristics. In baking, the proteins resistance to oil absorption is important in creating a light product. Results show decrease in the OHC of the seven investigated meal samples which indicates they could not be used in meat systems, sausages and doughnuts.

Gelation

Is important in comminuted meats as is a protein's emulsifying capacity. It is reported as the lowest concentration of protein that remained as a stable gel after 30 minutes at room temperature. The gelling property of all the seven meal samples resulting from the seven mixtures, remained the same as the defatted soybean meal (control). Samples that gel at 2% concentration are supposed to have a good gelling property. Circle et al. (13) reported that soy products gelled at concentrations as high 8- 10%.

Emulsifying Capacity

Emulsifying and film forming ability of plant proteins is essential for those proteins to perform well in meat systems, Also a protein's ability to form emulsions is critical to their applications in mayonnaise, salad dressing, milks, frozen desserts. The emulsifying capacity of the defatted soybean meal

and the seven investigated meals were in favor of their use in meat systems ,mayonnaise, and salad dressing. The emulsifying capacities ranged from 41.8 for meal resulting from autoclaved mixture III to 61.1(ml oil / 100 mg protein) for meal of dry heated mixture I.

Karel (20) reported that lipoproteins are potentially able to form strong films around oil droplets, and actually some lipoproteins ,including those of eggs,are excellent emulsifiers.

CONCLUSION

The presence of lipid-protein complexes associated with the meal protein causes changes in the isoelectric precipitation pattern of the protein.Changes in the electrophoretic pattern of the protein was observed. The nitrogen solubility index, water holding capacity, and oil holding capacity are damaged due to the presence of the lipid-protein complexes, while the gelation and emulsifying properties are improved.

REFERENCES

1. Pokorny J., Smidrkalva E., Zwain H. and Janicek G., *Nahrung* : 20, 707, 1976.
2. Pokorny J., Moravkova E.N., and Alexova H. , " Proceedings of 16th ISF Congress", 603, Budapest, 1983.
3. Pike O.A. and Peng I.C., *J. Food Science* : 53, 428, 1988.
4. Kamat V.B., Graham G.E., and Davis M.A.F., *Cereal Chem.*: 55, 295, 1987.
5. Abd El-Motaal E.A., Helmy H.E., Taha F.S. and Shoeb Z.E. , " Proceedings 47th Oilseed Conference, New Orleans, LA, March 1998.
6. Helmy H.E., Abd El-Motaal E.A., Shoeb Z.E. and Taha F.S., " Proceedings 47th Oilseed Conference, New Orleans, LA, March 1988.
7. EL-Nockrashy A.S., Mukherjee K.D. and Mangold H.K., *J. Agric. Food Chem.*: 25, 193, 1977.
8. Taha F.S., Abbasy M., El-Nockrashy A.S. and Shoeb Z.E., *J. Sci. Food Agric.*: 32, 166, 1981.
9. Laemmli U.K., *Nature*: 227 , 680, 1970.
10. A.O.C.S. METHOD Ba.11-65 From "Soybeans Chemistry and Technology"
Volume 1: Proteins P:
451, AVI publishing Inc., Westport, 1978.
11. Child E.A. and Forte J.F. , *J. Food Sci.*: 41, 652 , 1976.
12. "Soybeans Chemistry and Technology" , volume I: Proteins., ed. Smith A.K. and Circle S.J. , P:425, AVI Publishing Inc. , Westport, Connecticut 1978.
13. Circle S.J., Meyer E.N. and Whitney R.W., *Cereal Chem.*: 41 , 157, 1964.
14. Swift C.E., Lockett C. and Fryer A.J., *J. Food Tech.* : 15, 468, 1961.
15. Narayan K.A. and Kummerow F.A., *J. A.O.C.S.*: 40, 339, 1963.
16. Sanger F., *J. Biochem.*: 39, 507, 1945.

17. Izzo M.T. and Ho C.T., Cereal Chem.: 66, 47, 1989.
18. Wolf W.J. "Soybeans Chemistry and Technology", VOLUME 1: Proteins ,
Ed. Smith A.K. and Circle S.J. ,AVI Publishing Co. Inc., Westport,
Connecticut, 1978.
19. Finch R., Rev. in Food Techn.: 1, 519, 1970.
20. Karel M., J. Food Sci.: 38, 756, 1973.

Table 1 : Functional Properties of Meal Protein Resulting from The investigated Mixtures .

Functional Properties	I	II	III	IV	V	VI	VII	SM
Nitrogen Solubility Index (%)	2.1	1.9	1.5	3.5	9.4	2.1	1.9	13.2
Water Absorption Capacity (%)	180.0	200.0	170.0	250.0	230.0	220.0	190.0	260.0
Oil Holding Capacity (g oil/g meal)	2.4	2.7	2.1	1.9	2.5	1.9	1.9	2.7
Gelation (% concentration)	2.0	2.0	2.0	2.0	2.0	3.0	2.0	2.0
Emulsifying Capacity (ml oil/ 100 mg protein)	61.1	52.2	41.8	47.6	50.2	43.3	48.8	49.0

SM = defatted soybean meal (control)

Figure1 Isoelectric Precipitation Curves of Defatted Soybean Protein ,Meal Protein Resulting from Dry heated Mixture I and Moist Heated Mixture II .

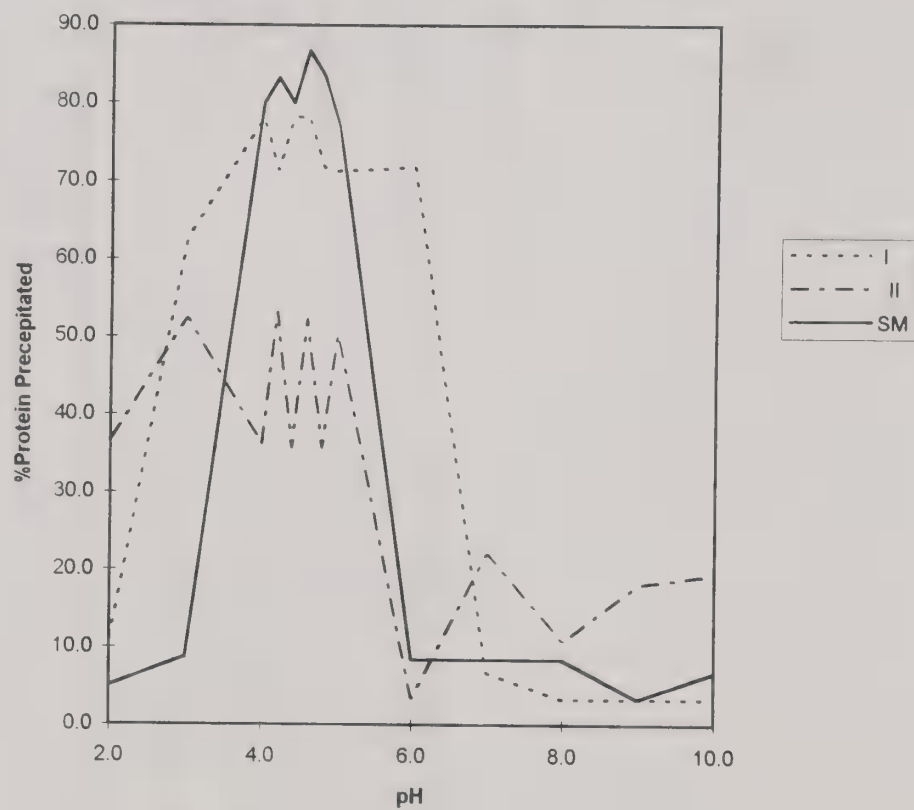
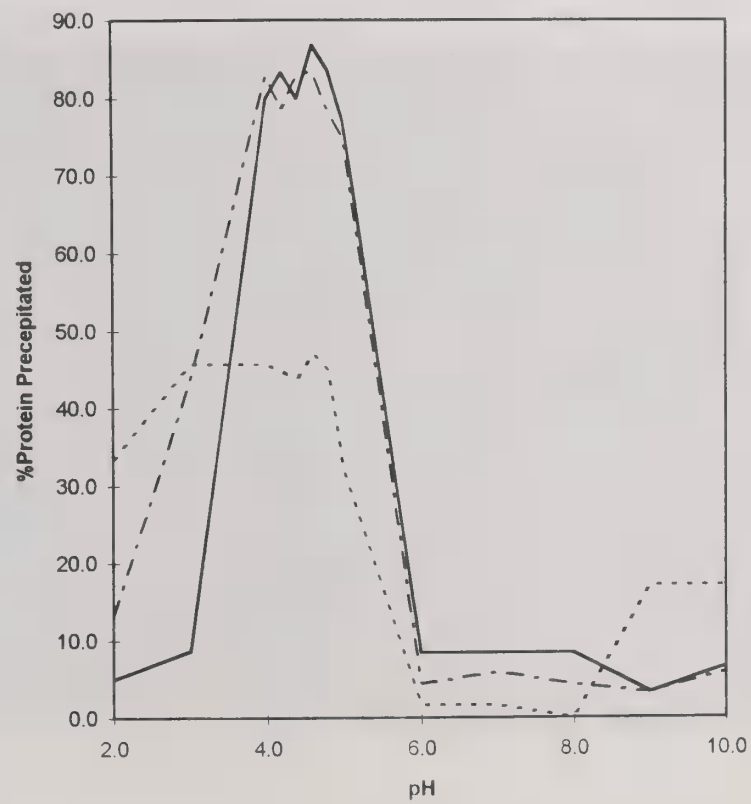


Figure 2 Isoelectric Precipitation Curves of Defatted Soybean Protein, Meal Protein Resulting from Autoclaved Mixture III and Stored Mixture IV .



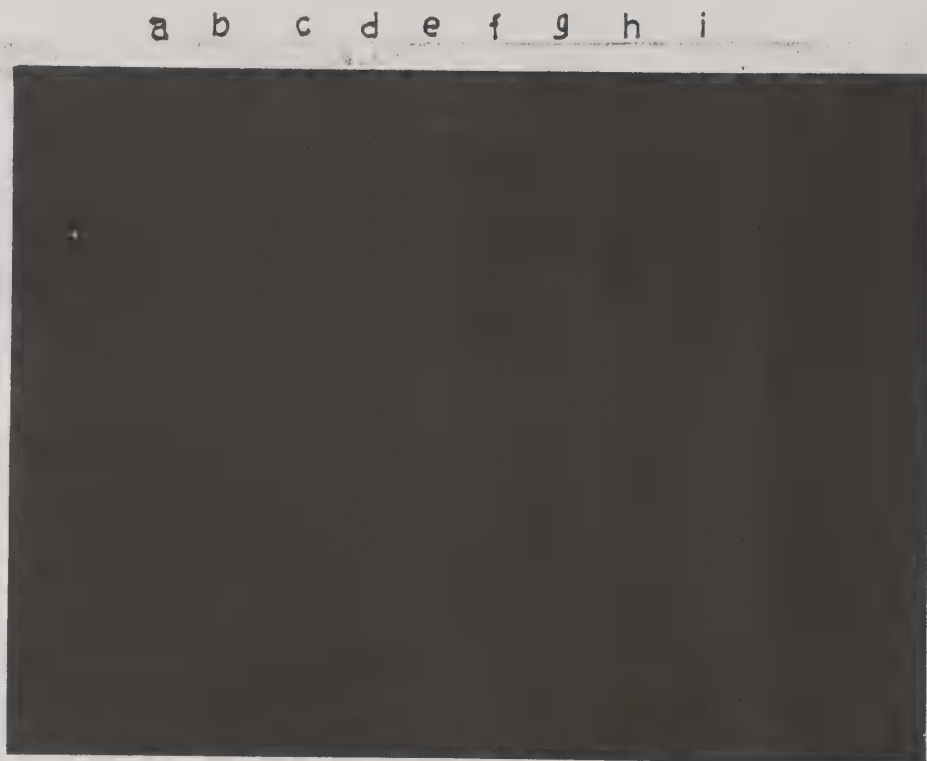


Figure (3) SDS - PAGE gel of treated soybean meals and untreated soybean meal .
a) Markers (MW range : 66-29 Kilo dalton); b) I ; c) V ;
d) III ; e) VII ; f) VI ; g) SM ; h) IV
I) II

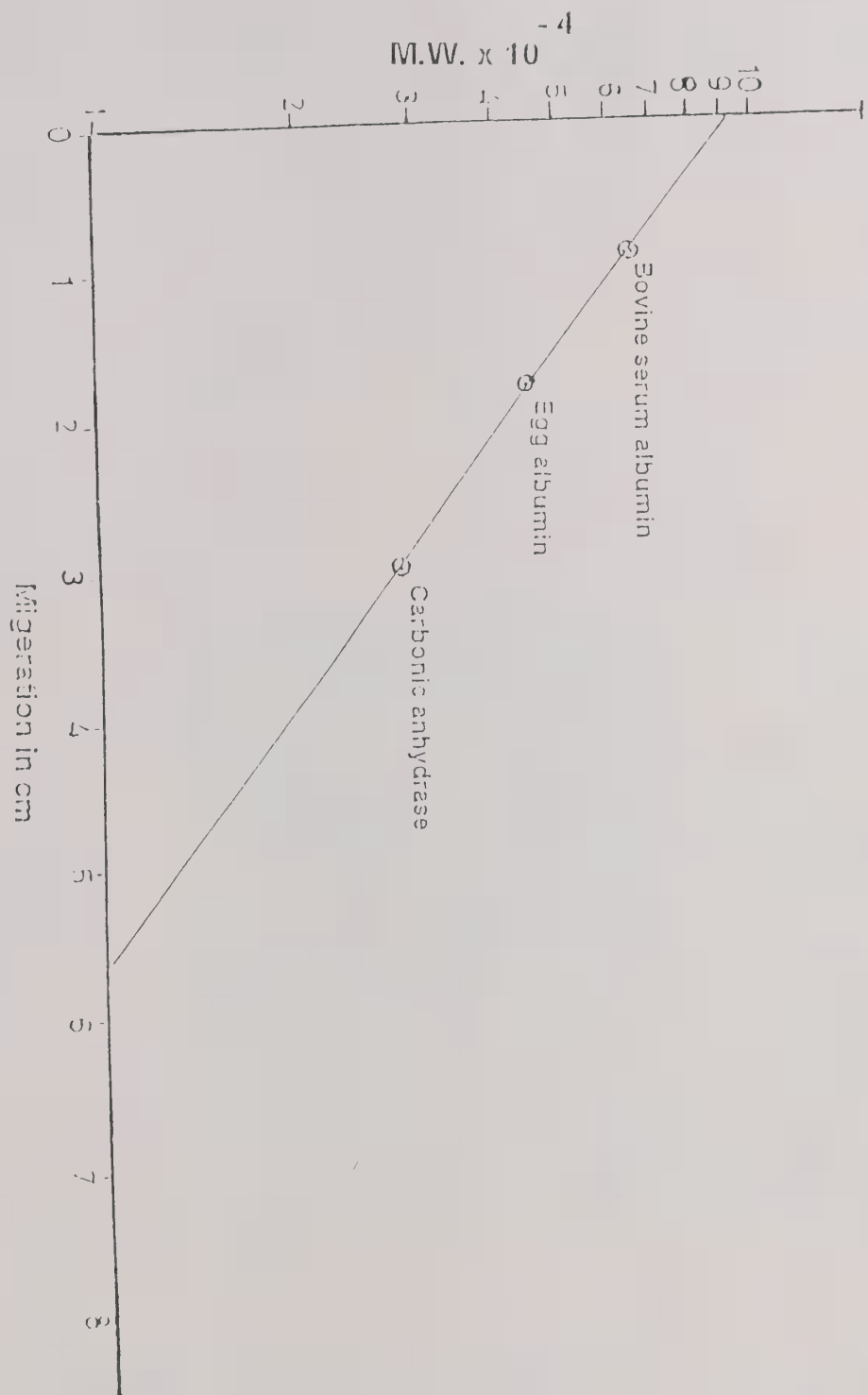


Figure (4) : Standard Curve of Molecular Weight of Protein Subunits .

*The Crystal and Molecular Structure
of the Gossypol Triacetic Acid Clathrate*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

The crystal and molecular structure of the gossypol triacetic acid clathrate.

Michael K. Dowd^a and Leonard M. Thomas^b

^aSouthern Regional Research Center, ARS, USDA, New Orleans, LA and

^bDivision of Science and Mathematics, Phillips University, Enid, OK

The crystal and molecular structure of the gossypol triacetic acid clathrate ($C_{30}H_{30}O_8 \cdot 3C_2H_4O_2$) was determined. Large, transparent, yellow, rectangular prisms were formed by crystallization from acetone. Solutions were prepared by adding 0.5 g of gossypol acetic acid to 3.0 ml of acetone at room temperature, and crystallization occurred by storing the solutions at 4 °C. An opaque layer of amorphous gossypol formed over the crystal surfaces within hours after removal from the solution, indicating that the crystals were unstable in air.

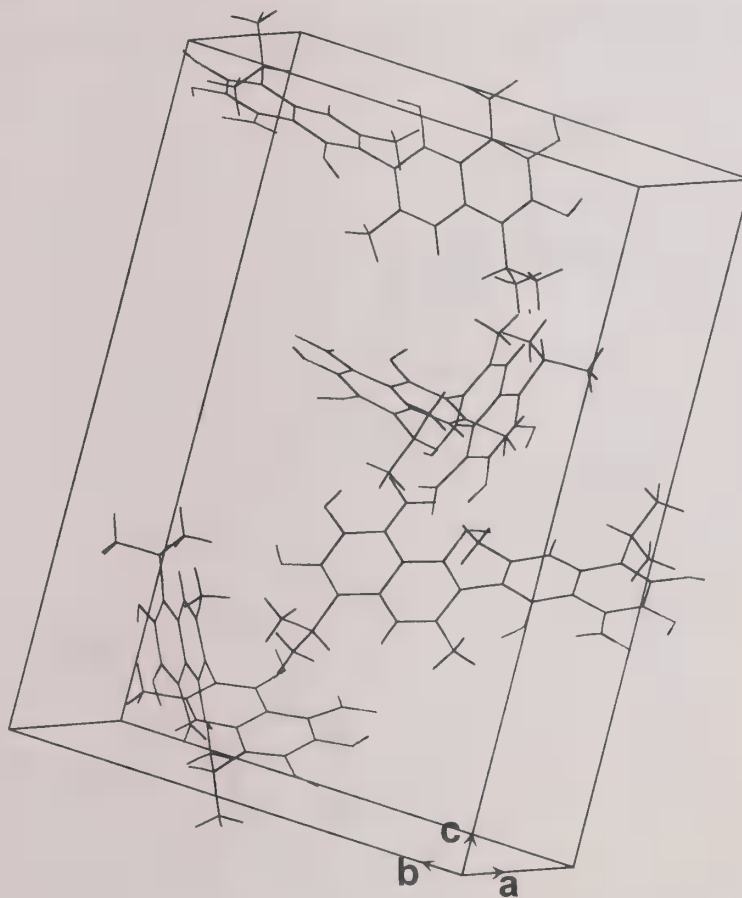


Figure 1. Unit cell of the gossypol acetic acid clathrate. The 12 molecules of acetic acid were removed for clarity.

Crystals were studied by X-ray diffraction at 213 K. The mounted crystal was coated with a thin layer of oil, which stabilized the structure sufficiently to collect diffraction data. The packing was orthorhombic with a $P2_12_12_1$ space group. Four molecules of gossypol and 12 molecules of acetic acid formed the unit cell with dimensions $a = 9.0208(7)$ Å, $b = 17.4884(10)$ Å and $c = 24.358(2)$ Å. The structure was refined to an R-factor of 0.0536 with the SHELXL-93 program.^{1,2} The orientation of the gossypol molecules within the unit cell is shown in Figure 1.

The gossypol molecules were all aldehyde tautomers, and were aligned along the c -coordinate axis with the bridged naphthalene rings close to perpendicular (The angle between the least-squared best estimate of the naphthalene ring planes was 88.3°). Open regions were filled with acetic acid molecules, and there were several hydrogen bonds between the acetic acid and gossypol molecules. The calculated density of the crystal was relatively low (1.208 g/cm^3). Substantial thermal motion was associated with the acetic acid molecules, and it is likely that these molecules are "dynamically disordered" at room temperature. This disorder probably contributes to the observed instability of the crystals by promoting the sublimation of the acetic acid molecules from the crystal surfaces outside the crystallization solution. An ORTEP plot (Oak Ridge Thermal Ellipsoid Plot) of the asymmetric unit is shown in Figure 2.

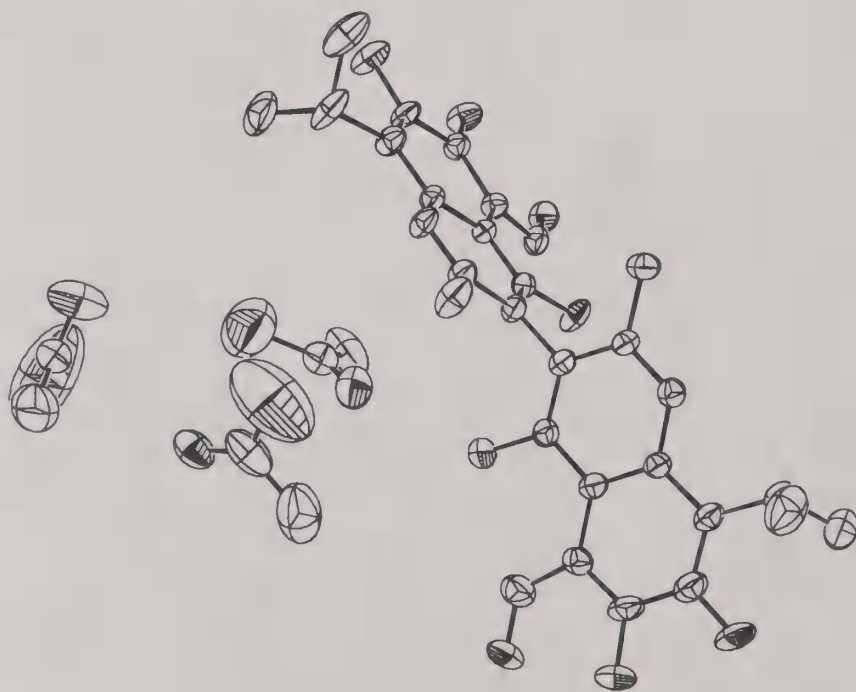


Figure 2. ORTEP plot of the asymmetric unit of the gossypol triacetic acid clathrate. Ovals represent the 50% probability volumes for the individual atoms.

Within individual crystals, all of the gossypol molecules were of one isomeric form. HPLC analysis and polarimetry of individual crystals confirmed this result. These tests indicated that both gossypol isomers crystallized from the supersaturated mother liquor but into separate crystals. Although gossypol crystallized from different solvents has different melting temperatures suggesting that polymorphism exists for the isolated molecule,³ the clathrate had generally been assumed to exist as the racemate with a one-to-one molar ratio of gossypol and acetic acid. The existence of large non-racemic crystals suggests that a method might be developed to separate small amounts of the individual gossypol isomers without resorting to derivatization to form diastereoisomers that can be separated by chromatography.

REFERENCES

1. Sheldrick, G.M. SHELXL93, Program for the refinement of crystal structures. Univ. of Göttingen, Germany, 1993.
2. International Tables for Crystallography, Vol. C, pp. 4.2.6.8 and 6.1.1.4.
3. Campbell, K.N., Morris, R.C., and Adams, R., J. Am. Chem. Soc., 59 1723-1728, 1937.

*Free Fatty Acids in Cottonseed and
Cottonseed Products: A Literature Survey*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

FREE FATTY ACIDS IN COTTONSEED AND COTTONSEED PRODUCTS: A LITERATURE SURVEY

E.J. CONKERTON, A.W. FRANK AND P.J. WAN
SRRC, ARS, USDA
NEW ORLEANS, LOUISIANA

INTRODUCTION

Fatty acids are essential constituents of cottonseed. At harvest time in mature seeds, they are found, primarily, in oil bodies as components of triacylglycerides. Some exist, however, as *FREE FATTY ACIDS* (*FFA*). To be graded prime quality seed with a quality index of 100, the *FFA* content of the extracted oil must be $<1.8\%$. This index, the basis for calculating the economic value of the seed, is reduced by 0.4 unit for each 0.1% *FFA* in excess of 1.8%. *FFA* contents $>5\%$ in freshly harvested cottonseed reduce the value of the seed and its use, whole, as a cattle feed. In addition, this *FFA* can cause problems in producing a high quality, edible cottonseed oil. The consequences of high *FFA* in cottonseed are well known, and, over the years, researchers have studied the intricacies of *FFA* formation in cottonseed. Also, they have worked to devise methods to prevent or retard *FFA* formation. The purpose of this literature survey was to assimilate the available knowledge in these areas and then determine:

1. Can this information provide the farmers, the ginners, the oilseed processors and the cattle feeders with efficient methods to control *FFA* formation?
2. If not, what research is needed?

The literature survey was suggested and supported by the National Cottonseed Products Association. Information collected from approximately 300 references included methods for *FFA* determination and formation of *FFA* in the field, at harvest, during ginning, during storage of the seed and during processing. Brief summaries of these topics will be presented.

METHODS FOR DETERMINATION OF FREE FATTY ACID

Methods for analysis of *FFA* in cottonseed are described in Table 1. While all give accurate results, none have the capability of providing a simple, rapid procedure useful in the field, at the gin or at storage sites.

FREE FATTY ACID FORMATION IN THE FIELD AND AT HARVEST

Low *FFA* is a criteria for good germination of cottonseed in the field. *FFA* reach a maximum during the first 2 - 6 days of seed germination but never exceed 3x's the initial *FFA* of the seed. *FFA* decreases as bolls develop and cottonseed mature on the plant, *i.e.* as triacylglycerides are formed. A *FFA* of 13% at 21 days after flowering drops to 1% at maturity. *FFA* differences in cottonseed do not appear to be associated with varietal differences. There may, however, be an association with seed size. Smaller seeds may contain more *FFA* than larger seeds grown and harvested under similar conditions. Environmental conditions and location do influence *FFA* of seed at harvest. A critical period in the maturation of cottonseed is 10 - 15 days after boll opening. If moisture content of the seed drops from 50% to 10% during that period, *FFA* should be $<2\%$. If weather conditions are wet and hot, the seed will not dry and *FFA* will rise.

Infection with field and storage fungi appear to be geographically localized. A clear relationship between infection (aflatoxin contamination) and *FFA* has not been defined.

Changes in cotton harvesting practices have been designed to improve cotton lint quality with minimum thought for the effects of these changes on seed quality. Plant growth regulators that reduce plant densities do not increase *FFA* and may have a beneficial effect. Minimal effects of defoliant on *FFA* were noted provided these chemicals are applied at appropriate times. No consistent effects of pesticides were noted.

FREE FATTY ACID FORMATION AT THE GIN

Seed cotton can be stored safely at the gin provided the modules are not compressed to densities >25 lbs per ft³ and seed moisture is <12%. All *seed cotton* must be protected by a tarp during storage. Seed damage can occur at the gin during the conveying, cleaning and drying of *seed cotton*. Damage increases as moisture and ginning rate increases. Artificial drying of damp *seed cotton* before ginning retarded but did not stop *FFA* formation during storage. A prediction of grade of seed received monthly at cotton gins was developed based on rainfall, temperature and relative humidity in the growing area during the months preceding receipt of the *seed cotton*. If proven practical, such information could provide the ginner and the crusher with quality estimates of seed as it is received at the gin.

FREE FATTY ACID FORMATION DURING STORAGE

Prime seed heated to 450°F for 4 min at the gin had a moisture content of 12.5% and contained only 3% *FFA* after 7 months unaerated storage. Similar unheated seed developed 6% *FFA* in four months.

Traditional storage at oilseed processing plants is in seed houses containing 30 - 1000 tons of seed. Aeration controlled the moisture content and temperature of a 40 ton lot of seed, but did not prevent a rise in *FFA*. *FFA* developed rapidly unless the seed had low initial moisture and *FFA* contents. Storage of smaller lots of seed showed similar results. Using data collected over a period of years, an equation was developed to predict the storability of cottonseed. The formula is based on the initial *FFA* and temperature of the seed.

Various chemicals have been applied to seeds before and after harvesting in attempts to prevent *FFA* formation in storage. Maleic hydrazide applied to cotton plants at defoliation retarded the development of *FFA* in ginned seed stored in small samples at 75 - 100% relative humidity for 28 days. Ethylene chlorhydrin applied at concentrations of 0.2% or higher (based on the dry weight of the seed) to artificially moistened cottonseed inhibited heating and *FFA* formation. Lower concentrations of the chemical stimulated *FFA* formation. The use of propionic acid (PA) to treat seed prior to storage has been studied by several investigators. At moisture levels below 20%, treatment with 1.5% PA based on the weight of the seed inhibited *FFA* formation during 180 days in sealed storage.

In laboratory studies, exposure of 20% moisture cottonseed to microwave heating prior to storage retarded the formation of *FFA* during storage. It was postulated that this treatment rapidly reduced seed moisture and destroyed lipolytic enzymes. Using 50 kg samples and a commercial microwave/convection heating unit, similar results were obtained. Seeds subjected to microwave heating will not germinate.

Although cottonseed meals are not normally stored, coarse meals of <12% moisture content were stored in sealed containers for 120 days before showing a rise in *FFA*.

FREE FATTY ACID DURING PROCESSING

Aqueous solvents (acetone or isopropanol water mixtures) favor separation of *FFA* from processed oil. Cooking of the meats and addition of alkali to the aqueous systems further improves oil quality. Alkali refining of crude cottonseed oil removes *FFA*, phosphatides and other non-glyceride components. While 0% *FFA* is desirable, 0.03% *FFA* in refined oil is satisfactory to produce an odorless oil by steam deoderization.

CONCLUSIONS

Data indicate that low moisture, low *FFA* seed can be stored safely. Wet seed should be dried as rapidly as possible. Drying temperatures and conditions that inactivate lipolytic enzymes are most effective in retarding *FFA* formation. Research is needed to develop rapid, simple methods of *FFA* analysis; efficient, practical seed drying techniques and seed quality management systems.

TABLE 1: METHODS FOR ANALYSIS OF FREE FATTY ACIDS IN COTTONSEED

All methods use oil extracted from dehulled, ground cottonseed.

TYPE OF ANALYSIS	COMMENTS
TITRATION AOCS OFFICIAL METHOD Aa 6-38	An International Standard Requires 200g of seed Measures <i>FFA</i> in oil, calculated as oleic Tedious and time-consuming
COLORIMETRIC	Requires a few milliliters of oil contg. <5mg oleic acid Requires reaction with cupric acetate Requires a centrifuge and a colorimeter Rapid, not well tested as a replacement for titration
THIN LAYER CHROMATOGRAPHY*	Separates <i>FFA</i> from other components Requires a densitometer for quantitation
GAS LIQUID CHROMATOGRAPHY	Requires esterification of <i>FFA</i> to methyl esters(FAME) Requires a gas chromatograph with FID detection Sum of FAME equal % <i>FFA</i> Results must be converted to %-as-oleic
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY	Requires derivatization to phenacyl esters Requires a HPLC with UV detection Sum of esters equals % <i>FFA</i> Results must be converted to %-as-oleic
COLUMN CHROMATOGRAPHY	Separates <i>FFA</i> from other components Requires quantitation by one of the above methods

* All chromatographic methods require <10 g of seed.

*Characterization of Lipid-Protein Complexes
from Developing Tung Seeds: Identification of
Potential Intermediates in Storage Oil Biogenesis*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Characterization of Lipid-Protein Complexes from Developing Tung Seeds: Identification of Potential Intermediates in Storage Oil Biogenesis

John M. Dyer¹, Alan R. Lax¹, Dorselyn C. Chapital¹, Fuqiang Tang^{1,2}, Ding S. Shih², and Armand B. Pepperman¹

¹USDA-ARS-SRRC, 1100 Robert E. Blvd., New Orleans, LA 70124

²Department of Biochemistry, LSU, Baton Rouge, LA 70803

(Extended Abstract)

INTRODUCTION

Plant storage oils are used throughout the world for both nutritional and industrial purposes. The properties of the oils are largely governed by the fatty acid constituents, which may be highly enriched in unusual fatty acids. For example, the oil produced from the tung tree is composed of approximately 80% eleostearic acid, which is a conjugated trienoic fatty acid. Eleostearic acid is easily oxidized when exposed to air, resulting in crosslinking of triglyceride molecules and formation of tough, chemically resistant films. This property makes tung oil a useful commodity in industrial applications that require a "drying oil."

The goal of our research is to identify the enzymes and proteins required for tung oil synthesis. An understanding of the molecular events is the first step towards the production of drying oils in other systems. For example, genes could be transferred to other plants for production of drying oils *in vivo*, or recombinant enzymes could be produced for modification of existing oils *in vitro*. We previously identified a subcellular fraction of developing tung nuts that was enriched in mature tung triglycerides and only a few proteins. In the current study, experiments were conducted to learn more about these lipid-protein complexes and characterize the nature of the proteins and their possible relationship to eleostearic acid synthesis.

METHODS

Analysis of plant material and preparation of subcellular fractions. Tung nuts were collected from the American Tung Oil Corporation in Lumberton, MS, from May to Sept., 1995. The chemical contents of tung seeds, including percent water, nitrogen, oil, and eleostearic acid, were determined. Tung seed lysates were prepared from seeds collected on July 26, Aug. 2, Aug. 16, Aug. 30, Sept. 13, and Sept. 27 to study changes in protein content during the period of active tung oil synthesis. Lipid-protein complexes were purified by differential centrifugation of the tung lysates. The final step of purification included dilution of the sample to reduce sucrose density to 1% and centrifugation for 2 h at 104,000xg to pellet the lipid-protein complexes. This fraction will be referred to hereafter as the 2 h pellet.

Characterization of the protein content in the lipid-protein complexes. Changes in protein content of the 2 h pellet fraction during seed development were monitored by SDS-PAGE, Coomassie Brilliant Blue R250 staining, and scanning densitometry (BioRad Model GS-700 Imaging Densitometer). Structural features of the three major proteins that correlated with eleostearoyl synthesis (p79, p55, and p39) were characterized by digestion of the 2 h pellet with trypsin. Products of the reactions were analyzed by SDS-PAGE. Digestions were also performed in the presence of 2.5% Triton X-100 to reveal protein domains that interacted with lipid. The 2 h pellet was also extracted using buffer conditions known to dissociate peripheral membrane proteins (1 M NaCl or 0.1 M Na₂CO₃, pH 11). Finally, proteins in both the 2h pellet fraction and mature oil bodies were purified and microsequenced. Proteins were separated by SDS-PAGE, transferred to PVDF membrane, and stained using Coomassie Blue. Bands corresponding to the proteins of interest were excised and their N-terminal amino acid sequences were determined by Dr. Richard Cook, Baylor College of Medicine.

Characterization of particle morphology and lipid content in the 2 h pellet fraction. Lipid particles in the 2 h pellet fraction were mounted on copper grids, negatively stained using 1% ammonium molybdate, and viewed using a Phillips CM 120 electron microscope. The lipid classes in the 2 h pellet were determined by using TLC. Duplicate silica gel G plates were developed using mobile phases specific for neutral lipid separation (hexane:diethyl ether:acetic acid (80:20:1)) or phospholipid separation (chloroform:methanol:ammonium hydroxide (33% w/v):water (65:35:5:2.5)). After spraying with 0.2 M cupric acetate in 8% phosphoric acid, lipid spots were detected by charring for 15 min at 120°C. After cooling, lipids were quantified by scanning densitometry and comparison to lipid standard curves.

RESULTS

The abundance of three proteins in the 2h pellet fraction correlates with the synthesis of eleostearic acid. Previously, we identified lipid-protein complexes in developing tung nuts that were enriched in eleostearoyl lipids and contained only a few proteins. To investigate the relation of these proteins to the synthesis of eleostearic acid, lipid-protein complexes were isolated from tung nuts collected before, during, and after the active stages of eleostearic acid accumulation. The subcellular fraction enriched in the lipid-protein complexes is referred to as the 2 h pellet.

Analysis by SDS-PAGE of proteins in the 2 h pellet fraction demonstrated that the protein profile changed considerably throughout the course of seed development (Fig. 1). Three major proteins of approximately 79, 55, and 39 kDa (p79, p55, and p39, respectively) increased in abundance during eleostearic acid active accumulation and then decreased rapidly after the Aug. 30 date. Two other proteins of approximately 46 and 29 kDa increased at this time and remained at high levels. The levels of these two proteins were tightly coupled (Fig. 1),

suggesting a close biosynthetic relationship. Measurement of oil content in the developing seeds indicated that eleostearic acid increased to the highest point on Aug. 30 and then leveled off (Fig. 2). Comparison of the kinetics of eleostearoyl synthesis and level of proteins in the 2 h pellet demonstrates a positive correlation between amount eleostearic acid and the abundance of the p79, p55, and p39 proteins (Fig. 2).

Characteristics of the proteins in the lipid-protein complexes. Mapping the structural features of the proteins in the 2 h pellet fraction by trypsin digestion revealed that a small domain of approximately 15 kDa could be cleaved from p79, resulting in accumulation of a 64 kDa fragment. Inclusion of Triton X-100 in the reaction increased the susceptibility of p79 to trypsin digestion, suggesting that the 64 kDa domain was protected by lipids. The digestion of p55 and p39 remained the same in the presence or absence of detergent, suggesting little or no lipid protection. Extraction of the lipid-protein complexes using high salt or pH led to dissociation of the proteins from the lipid-protein complexes, suggesting that the proteins were peripherally associated with the lipids.

Several of the proteins in the 2 h pellet fraction were purified and their N-terminal amino acid sequences were determined. The p79 protein exhibits sequence similarity to plant subtilisin-like proteases. Another protein found in the pellet (p21), which did not correlate with eleostearoyl synthesis, had sequence similar to 11S globulin-type seed storage proteins. Tung oleosin protein was also purified and was very similar to other known oleosin sequences.

Lipid-protein particle morphology and lipid content. Electron microscopic analysis of the 2 h pellet fraction revealed that there were two general populations of particles present. One population was fairly small and uniform in size, with an average diameter of 84 ± 18 nm (range 70-105 nm). These smaller particles likely represent contamination of the lipid-protein

complexes with microsomal membranes, which typically range in size from 50-150 nm. The second population was larger and more variable in size, with an average diameter of 280 ± 70 nm (range 210-420 nm). These particles are larger than typical microsomes, yet smaller than mature oil bodies ($\sim 1,000$ nm). Analysis of lipid content by thin layer chromatography indicated that the 2 h pellet was highly enriched in neutral lipid content ($\sim 75\%$).

DISCUSSION

The data presented here suggest that the lipid-protein complexes may represent intermediates in the biosynthetic pathway of mature oil bodies. This fraction contains several proteins whose accumulation coincides very closely with eleostearate biosynthesis. Morphological analysis demonstrates that these complexes are larger than typical microsomal membranes, yet smaller than mature oil bodies. Finally, the complexes are highly enriched in neutral lipids containing eleostearic acid. Yatsu and Jacks have previously observed spherosomes in developing tung seeds that are similar in size to the particles described here (Amer. Tung News (1969) 20:6-7). This raises the possibility that the lipid-protein complexes purified in the 2 h pellet fraction represent distinct subcellular entities rather than adventitious lipid-protein complexes generated during homogenization of the tissue. We are currently trying to resolve this issue by performing immuno-electron microscopy using antibodies raised to p79. In addition, more sequence data and further experimentation will be required to define the true function of p79, as well as the other two proteins that correlate with eleostearoyl synthesis. It is hoped that one of these proteins will show similarity to existing fatty acid desaturases and function directly in the synthesis of eleostearic acid.

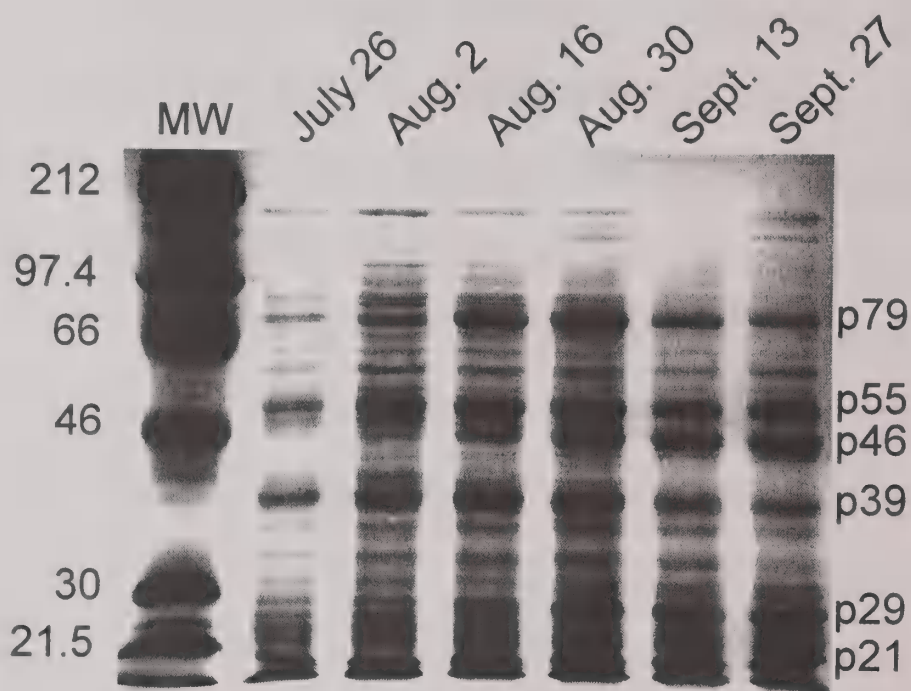


Figure 1. Changes in protein content of the 2 h pellet fraction during tung seed development. Proteins were separated using SDS-PAGE and stained with Coomassie Blue. The sizes of molecular weight standards are shown to the left. The approximate sizes of major proteins in the 2 h pellet are shown to the right.

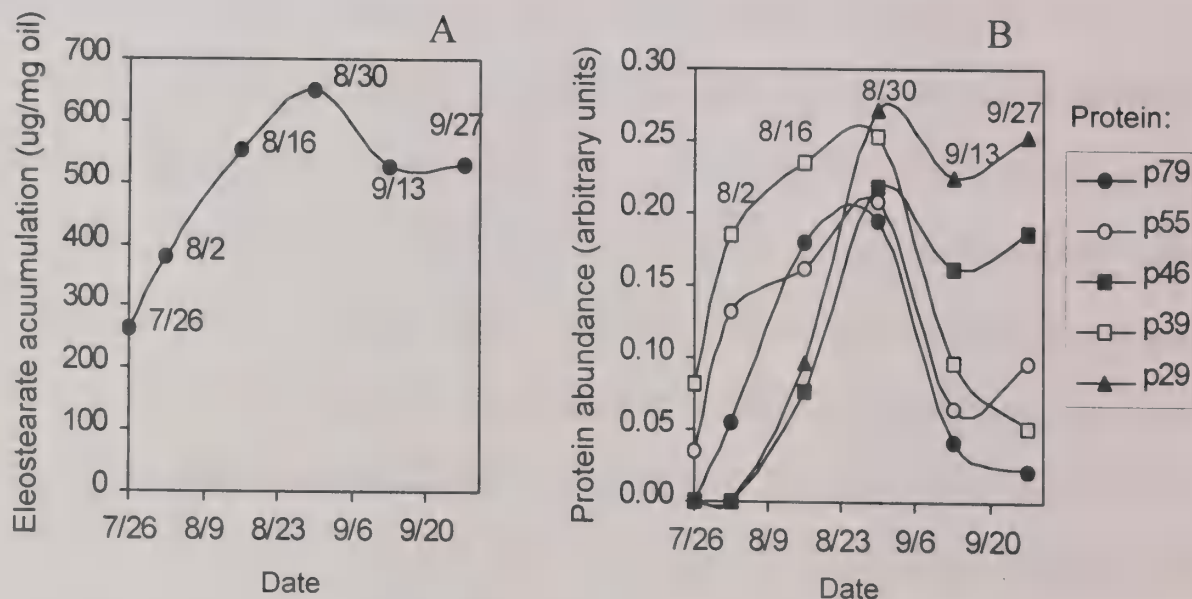


Figure 2. Comparison of eleostearoyl content (A) to protein abundance in the 2 h pellet fraction (B).

*Cloning of a Full-Length Tung Seed
cDNA Homologous to Endoplasmic Reticular
Fatty Acid Omega-3 Desaturases*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Cloning of a Full-Length Tung Seed cDNA Homologous to Endoplasmic Reticular Fatty Acid Omega-3 Desaturases

Fuqiang Tang^{1, 2}, John M. Dyer¹, Alan R. Lax¹, Ding S. Shih², Dorselyn C. Chapital¹, Armand B. Pepperman¹

¹Southern Regional Research Center, U.S. Department of Agriculture, 1100 Robert E. Lee Blvd., New Orleans, LA 70124

²Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803

Abstract

The primary objective of this research was to clone a fatty acid desaturase cDNA involved in tung oil biosynthesis. For this, PCR was conducted using tung genomic DNA and primers based on conserved regions of known plant fatty acid desaturases. A 766-bp fragment that exhibited high homology to other plant fatty acid desaturases was cloned and sequenced. To obtain the full-length cDNA of the desaturase, RT-PCR and RACE were conducted with primers designed according to the cloned genomic DNA sequence. Template cDNA was obtained by reverse transcription of mRNA extracted from tung seeds actively synthesizing tung oil. We have cloned a cDNA, designated Tndes1, which represents a novel tung desaturase expressed during active synthesis of tung oil. Tndes1 contains 1358 nucleotides, which includes a 5' untranslated region of 38 nucleotides, an open reading frame of 1161 nucleotides encoding 387 amino acids, a stop codon, 139 nucleotides of 3' untranslated sequence, and a 16-nucleotide polyadenylated tail. The amino acid sequence is predicted to be a membrane protein that has 4 transmembrane

helices by SOSUI (Prediction of Transmembrane Segments, Mitaku Lab., TUAT). The amino acid sequence of Tndes1 showed the greatest homology to endoplasmic reticular omega-3 desaturase from rapeseed (68% identity, GenBank [L01418](#)), *Arabidopsis thaliana* (67% identity, GenBank [L22931](#)) and soybean (66%, GenBank [L22964](#)) (Fig. 2). This is the first documentation of the isolation and complete sequencing of a gene involved in fatty acid biosynthesis in tung.

Introduction

Eleostearic acid is a conjugated fatty acid which makes tung oil a commercially valuable “drying oil”, important in paint and coating industries. However, little is known about the biosynthesis of eleostearic acid. One approach to understand the synthesis is to identify the genes and the enzymes associated with eleostearoyl biosynthesis and metabolism. Fatty acid desaturases of plants have received considerable attention because their function determines the nutritional and industrial quality of plant seed oils (Battey et al. 1989). The objective of this research was to isolate tung fatty acid desaturase genes from seed tissue that was actively involved in eleostearate synthesis. We report here the cloning of a cDNA encoding a novel fatty acid desaturase that appears to be an endoplasmic reticular fatty acid ω -3 desaturase.

Material and Methods

Materials Tung nuts were harvested from the fields of American Tung Oil Corporation in Lumberton, MS during the period of active tung oil synthesis in 1997. The hulls were removed and the seeds were immediately frozen in liquid N₂ and stored at -80 °C. Young tung leaves were frozen in liquid N₂ and stored immediately at -80 °C.

Isolation of DNA from tung leaves Tung leaf DNA was isolated by a modified method of Doyle and Doyle (1987). Briefly, 10g of frozen tung leaves were ground in liquid nitrogen using a mortar and pestle and extracted with 20 ml 2XCTAB (2% CTAB, 1.4 M NaCl, 2% PVP, 0.2% β-Mercaptoethanol, 100 mM Tris pH8.0) buffer. The homogenate was incubated 1hr at 65°C and spun at 9,000 rpm for 10 min. The supernatant was extracted by adding an equal volume of chloroform:isoamylalcohol (24:1, vol/vol). After centrifugation at 9,000rpm for 15 min, the upper aqueous phase was saved and DNA was precipitated by adding 2/3 volume of ice-cold isopropanol. After centrifugation at 3,500 rpm for 5 min, the DNA pellet was washed with 70% ethanol, air dried briefly and resuspended in 0.5 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA., pH 8.0).

mRNA isolation and cDNA library construction Total RNA was isolated from tung seeds during active synthesis of eleostearic acid by the method of Bugos et al. (1995). mRNA was then isolated using Poly(A) Pure mRNA Isolation Kits according to the manufacturer's instruction (Ambion). cDNA was prepared from ca. 5mg mRNA, using

Marathon cDNA Amplification Kit, according to the manufacturer's protocol (Clontech). A cDNA library was constructed using λ TriplEx library construction kit by Clontech.

PCR amplification of nucleotide sequence homologous to ω -3 desaturase Two sets of degenerate oligonucleotides were designed based on the most conserved sequences among the known plant ω -3 desaturase sequences. The two sets have the following sequences:

5'-GCTTGTTGGACTGCAATGGC-3' and 5'-GGGATYTGHHGGGAARAGATGATG-3'. PCR with leaf genomic DNA was performed in a 50- μ l volume and consisted of 10 μ M sense and antisense oligonucleotides, 1 μ g genomic DNA, 5mM MgCl₂, 0.2 mM dNTPs, 1X Taq reaction buffer (Promega) and 2.5 units of Taq polymerase (Promega). Temperature conditions for PCR amplification were 5 min at 96 °C, 75 °C for 3 min, and 40 cycles of 30 seconds at 96 °C, 1 min at 58 °C and 1 min at 72 °C. This was followed by an additional 15 min extension at 72 °C. PCR fragments of approximately 766 base pairs were cloned into TA vector by using TA Cloning Kit (Invitrogen), according to the manufacturer's protocol.

Rapid Amplification of cDNA Ends (RACE) and cDNA Library Screening To obtain a full-length cDNA, RACE was conducted with the cDNA template synthesized from tung seed mRNA. Specific primers were designed based on the tung genomic DNA sequence cloned and the conserved sequences of known desaturase cDNAs. The specific primer for 5'RACE is: 5'-TCCATTCCTTTCCGCGATACC-3'. The specific primer for 3'RACE and nested specific primer are 5'-TACTCTGTGCCTCTCCCTATGTTTGC-3',

and 5'-GAATTAGCCACAGGACTCACCATCAA-3' respectively. The amplifications were completed by using the 5'/3' RACE Kit, according to the manufacturer's protocol (Manheim Boehringer). The cDNA library was screened by PCR according to the manufacturer's protocol (Clontech).

DNA sequencing Plamid DNA was purified using the Wizard plus Minipreps DNA Purification System (Promega) and sequenced on both strands using SequiTherm ExcelTMII, Long-ReadTM DNA Sequencing Kit-LC (Epicentre Technologies) and 4000L Automated DNA Sequencer (LI-COR).

Results and discussion

Cloning of a tung genomic DNA sequence encoding a desaturase-related sequence

Using degenerate primers based on conserved regions of fatty acid desaturase available in BeneBank and tung genomic DNA, a 766 bp fragment was amplified by PCR. Alignment of the tung genomic DNA sequence with plant ω -3 desaturase cDNA sequences demonstrated that the amplified tung DNA fragment likely encoded a fatty acid desaturase gene. A 117 bp fragment at the 3' end of the sequence was found to be homologous to known desaturase cDNAs available in GeneBank (Fig. 1). The similarities between the homologous tung genomic DNA sequence and the cDNA sequences are greater than 80%. The most similar cDNA is castor bean desaturase, with 86% similarity.

Cloning of a full-length tung cDNA encoding an ω -3 desaturase To isolate a cDNA encoding a tung fatty acid desaturase, mRNA was isolated from tung seeds actively synthesizing eleostearic acid and reverse transcribed into cDNA. This sample was used as template in RACE experiments. 5'RACE and 3'RACE were conducted with specific primers designed based on the conservative sequence among the known plant fatty acid desaturase cDNA sequences and tung genomic DNA sequence cloned in this research. Fragments of 918 bp from 5'RACE and 817 bp from 3'RACE, with 264 bp overlapping sequence between the two fragments, were cloned. The overlapping regions were identical. The assembled sequence, designated Tndes1, is 1358 bp long, which was also cloned by screening cDNA library with PCR. Tndes1 includes a 5' untranslated region of 38 nucleotides, an open reading frame of 1161 nucleotides which encodes a 45-kDa polypeptide having 387 amino acids, a stop codon, 139 nucleotides of 3' untranslated sequence, and a 16-nucleotide polyadenylated tail. Alignment of the deduced amino acid sequence with endoplasmic reticular ω -3 desaturases from *Arabidopsis thaliana*, rapeseed, soybean and the plastidial castor bean fad7 sequence shows several regions that are identical and an overall similarity range from 66%-70%. Tndes1 amino acid sequence showed the greatest homology to endoplasmic reticular omega-3 desaturase from rapeseed (68% identity, GenBank [L01418](#)), *Arabidopsis thaliana* (67% identity, GenBank [L22931](#)) and soybean (66%, GenBank [L22964](#)) (Fig. 2). It shows 70% identity to castor bean plastid linoleic acid desaturase (fad7, 70%, GenBank [L25897](#)). However, it is much shorter than fad7 desaturase. The major difference between the primary structure of Tndes1 and other endoplasmic reticular desaturases occurs in a region near their amino termini. In this region the soybean desaturase contains 3 less amino acid than Tndes1.

Similarly, this portion of the rapeseed and *Arabidopsis thaliana* desaturase lacks 4 and 1 amino acid respectively relative to the Tndes1 desaturase. The alignment revealed three highly conserved histidine boxes as well as their spacing characteristics for membrane-bound desaturases (Fig. 2). These boxes have the general sequence of $\text{HX}_{2(3)}[\text{XH}]\text{H}$ (underlined sequences in Fig. 2) (Sperling et al. 1995). The distance between the first and second histidine boxes of the Tndes1 is 31 amino acids which is found in all membrane-bound desaturase, where the boxes are separated by 31 or 32 amino acids (Sperling et al. 1995). The deduced amino acid sequence contains a motif (DCGH) that is similar to EXXH motif, a part of the complex, di-iron-binding site in ribonucleotide reductase (Nordlund et al. 1990). The EXXH has been shown to be present in acyl carrier protein desaturase (Fox et al., 1993). It appears very likely that the Tndes1 gene encodes a functionally equivalent ω -3 desaturase in tung. Tndes1 amino acid sequence is of a membrane protein that has 4 transmembrane helices as predicted by SOSUI (Prediction of Transmembrane Segments, Mitaku Lab., TUAT) (Fig.3). An ER membrane retention signal, KKXX-like motif (KSKL) was found in the C-terminus of the peptide. The NH_2 -terminal region of Tndes1 desaturase, like other extraplastidial desaturases, does not exhibit the characteristics of an N-terminal signal peptide (PSOR, WWW Server for Analyzing and Predicting Protein Sorting Signals). However, the fad3 enzymes localized in the chloroplast typically have a chloroplast targeting leader sequence (Yadav et al. 1993). Microsomal ω -3 desaturases from *Arabidopsis thaliana* and rapeseed share the motif of two lys residues positioned three and five residues from the C terminus that is believed to be sufficient for retention of transmembrane ER proteins (Jackson et al., 1990). This motif is absent from the putative plastid homologs from all three species.

(Yadav et al. 1993). These characteristics suggest that Tndes1 encodes a membrane-bound protein located in the ER.

Conclusion

The cloning of Tndes1 represents the first isolation of a gene for putative membrane-bound endoplasmic reticular fatty acid desaturase from tung. The data which supports the endoplasmic reticular location is based on the similarity of this protein to other known ER ω -3 desaturase genes and genetic information. These enzymes have been difficult to analyze by conventional biochemical methods. The results presented here provide new information about fatty acid biosynthesis in tung seed actively synthesizing tung oil. The availability of the gene will greatly facilitate studies of the cellular localization, regulation, and catalytic properties of this important class of enzymes in tung seeds.

Reference:

- 1 Arondel,V., Lemieux,B., Hwang,I., Gibson,S., Goodman, HM, Somerville, CR (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in Arabidopsis Science 258: 1353-1355.
- 2 Battey, JF, Schmid KM, Ohlrogge, JB (1989) Trends Biotech. 7:122-125.

- 3 Bugos RC, Chiang VL, Zhang XH, Campbell ER, Podila GK, Campbell WH (1995) RNA isolation from plant tissues recalcitrant to extraction in guanidine. *BioTechniques* 19:734-737.
- 4 Doyle, JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of the fresh leaf tissue. *Phytochem. Bull.* 19:11-15
- 5 Fox BG, Shanklin J, Somerville CR, Munck E (1993) Stearoyl-acyl carrier protein δ -9 desaturase from *Ricinus communis* is a diiron-oxo protein. *Proc. Natl. Acad. Sci. U. S. A.*
- 6 Jackson Mr, Nillson T, Peterson PA (1990) Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticular. *EMBO J* 9:3153-3162.
- 7 Nordlund P, Sjoberg B-M, Eklund H (1990) Three-dimensional structure of the free radical protein of ribonucleotide reductase. *Nature* 345:593-598.
- 8 Sperling P, Schmidt H, Heinz E (1995) A cytochrome-b5-containing fusion protein similar to plant acyl lipid desaturases. *Eur. J. Biochem.* 232:798-805
- 9 Yadav NS, Wierzbicki A, Aegerter m, Caster CS, Perez-Grau L et al. (1993) Cloning of Higher Plant w-3 fatty acid desaturases. *Plant Physiol.* 103:467-476.
- 10 Van de Loo,F.J. and Somerville,C (1994) Plasmid omega-3 fatty acid desaturase cDNA from *Ricinus communis* *Plant Physiol.* 105 (1), 443-444.

Acknowledgments:

We would like to thank Anjel M-Guitroz for technical assistance and Edith J. Conkerton for helpful suggestions.

A

1 Tngenol	679	GGGATGGA	GTTATCTGAGAGGAG	GGCTTACAACCTCTTG	ATCGAGATTATGGTT
2 Soybean	1675	GGAATGGA	GTTATTTAAGAGGTG	GCCTCACCCTGTGG	ATCGTGACTATGGTT
3 Rape	884	GGAATGGA	GTTACTTGAGAGGAG	GACTTACAACATTGG	ACCGGGACTACGGAT
4 Castorbean	1396	GGCATGGA	GTTATCTAAGAGGAG	GGCTTACAACCCTTG	ATCGCGACTACGGAT
		** *****	*****	* ***** *	* * * * * * * * * * * * *

1 Tngenol	TGATCAATAACATCC	ACCATGATATTGGTA	CTCATGTCATTCATC	ATCTTTTCCCTCAAA
2 Soybean	GGATCTATAACATTC	ACCATGACATTGGCA	CCCATGTTATCCACC	ATCTTTTCCCCCAAA
3 Rape	TGATCAACAACATCC	ATCAGGACATTGGAA	CTCATGTGATACATC	ATCTTTTCCCTCAGA
4 Castorbean	GGATCAATAACATCC	ACCATGACATAGGAA	CCCACGTTATTCATC	ATCTCTTCCCTCAAA
	*****	* ***** *	* * * * * * * * * *	* * * * * * * * * *

1 Tngenol	TCGC	766
2 Soybean	TTCC	1762
3 Rape	TCCC	971
4 Castorbean	TCCC	283
	* *	

B

Deduced amino acid sequence of Tngenol:

GWSYLRGGLTTLDRDYGLINNIHHDIGTHVVIHHLFPQI
Histidine box 3

Fig. 1. Part of the amplified tung genomic DNA sequence is homologous to other known desaturase cDNA sequences(A). Tngenol, part of the amplified tung genomic DNA sequence. Rape, Arath, Soybean and castorbean refer to part of endoplasmic reticulum ω -3 desaturase cDNA sequences from rapeseed (Arondel et al 1992, soybean (Yadav et al. 1993) and plastid fad7 from castor bean (Van De Loo and Somerville et al. 1994) respectively:

Sequence 1: Tngenol	117 bp	Start of Pairwise alignments
Sequence 2: Soybean	117 bp	Sequences (1:2) Aligned. Score: 80
Sequence 3: Rapeseed	117 bp	Sequences (1:3) Aligned. Score: 82
Sequence 4: Castorbean	117 bp	Sequences (1:4) Aligned. Score: 86

The deduced amino acid sequence of Tngenol contains histidine box3 conserved among fatty acid desaturases (B).

castorbean MAAGCVLSECGRLRPLRIYRSRGFTSKTTNNLLKRLPDSKSYNLCSSFKVSSWSNSK

Tndes1 -----MKQQQYKDTPI L N G V N G F H A K E E-----EEEEFDLSNP PPFNI GOIRAAI
Rape -----M V V A M D Q R--S N V N G D S G A R---KEEGFDP SAQP PFKIGDIRAAI
Arath -----M V V A M D Q R--T N V N G D P G A G D R K K E E R F D P S A Q P P F K I G D I R A A I
Soybean -----M V K D T K P L A Y A A N N G Y Q Q K-G S S F D F D P S A P P P E K I A E I R A S I
castorbean QSNWALNVAVPVNVSTVSGEDDREREENFGIVNVDE-GKGFEFFDAGAPPPETLADIRAAI
* * * * *

Tndes1 PKHCWVKNPWRSLTYVFRDVVVVFALAAAAFYFN SWLF WPLYWFAOGTMFWAIFVLGHDC
Rape PKHCWVKSPLRMSYVTRDI FAVALAMAAVYFDSWFLWPLYWVAOGLFWAIFVLGHDC
Arath PKHCWVKSPLRMSYVVRDII AVAALAIAAVYVDSWELWPLYWAAOGLTFWAIFVLGHDC
Soybean PKHCWVKNPWRSLSYVLRDLVIAALVAAA IHFDNWLLWLIIYCPIOGTMFWALFVLGHDC
castorbean PKHCWVKNPWRMSYVLRDVVVVFGLAAVAAYFN NVVAVPWLYWF COGTMFWALFVLGHDC
***** * * . * * * * . * * * * * **** * * . *****
Histidine Box1

Tndes1 GHGSFSNNSSLN NVVGHL LHSSILVPYHGWRISHRTHQHNGHVEKDES WVLP EK IYKE
Rape GHGSFSDIPLL NSVVGHI LHSFILVPYHGWRISHRTHQHNGHV ENDES WVLP EK LYKN
Arath GHGSFSDIPLL NSVVGHI LHSFILVPYHGWRISHRTHQHNGHV ENDES WVLP ERVYKK
Soybean GHGSFSDSPLL NSLVGHI LHSILVPYHGWRISHRTHQHNGH IEKDES WVLP TEKIYKN
castorbean GHGSFSNNPKL NSVVGHL LHSSILVPYHGWRISHRTHQHNGHV ENDES WHPL SEKIFKS
***** ** . *** * * * ***** * * * * * * * . * * . *
Histidine Box 2

Tndes1 MDLSTRILRYSVLPMPFALPFYLWWRSPGKEGSHFNPN SDF FAPHERKAVLT SNFCFSIM
Rape LPHSTRMLRYTVPLEMLAYPIYLWYRSPGKEGSHFN PYSSLFAPSERKLIATSTTCWSIM
Arath LPHSTRMLRYTVPLEMLAYPLYL CYRSPGKEGSHFN PYSSLFAPSERKLIATSTTCWSIM
Soybean LDSMTRLIRFTVPFPLFVYPIYLF SRSPGKEGSHFN PYSNLFP SERKGIATSTLCWATM
castorbean LDNVKTLRFSLFPMLAYPFYLWRSRPGKKGSHFHDSGLFVPERKDITSTACWTAM
. * . *
* * . *
* * . *

Tndes1 ALLLYSCFEVGPVOVLKFYGIPYLVFMWLD FVTYLMHHGHGEEKLPWYRGKEWSYL RGG
Rape LATLVYLSFLVD PVTVLKVYGVPYII FVMWLD AVTYLHHHGHGDEKL PWYRGKEWSYL RGG
Arath FVSILALSFEVGFELAVLKVYGVPYII FVMWLD AVTYLHHHGHGDEKL PWYRGKEWSYL RGG
Soybean FSLLIYLSFITSP LLVLKLYGIPYWI FVMWLD FVTYLMHHGHGDEKL PWYRGKEWSYL RGG
castorbean AALLVYLNFSMGPMOMLKL YGIPYWI FVMWLD FVTYLMHHGHGDEKL PWYRGKAWSYL RGG
* . * * * . *

Tndes1 LQTVDRDYGW INNIHHDIGTHVIHHLFPQIPHYHLIEATKAAPVLGKY YREP KKSGPFF
Rape LTTIDRDYGI FN NIHHDIGTHVIHHLFPQIPHYHLVDATRAAKHVLGRYYREPKTS GAIP
Arath LTTIDRDYGI FN NIHHDIGTHVIHHLFPQIPHYHLVDATAAKHVLGRYYREPKTS GAIP
Soybean LTTVDRDYGWIYNIHHDIGTHVIHHLFPQIPHYHLVEATQAAPVLGDYYREPE RSAPLP
castorbean LTTLD RDYGW INNIHHDIGTHVIHHLFPQIPHYHLVEATEAAPVMGKY YREP KKSGP LP
* * . *
Histidine Box 3

Tndes1 FHLSNLVRSMSEDHYVSDIGDIVFYQTD PD DIYKV DKS KLN--
Rape IHLVESLVASIKKH DYVSDTG DIVFYETDP DLVYASDK SKIN
Arath IHLVESLVASIKKH DYVSDTG DIVFYETDP DLVYASDK SKIN
Soybean FHLIKYLIQS MRQDH FVSDTG DVVYYYQ TDSL LLSQRD----
castorbean LHLGLSLVRSMKEDHYVSDTG DVVYYYQ DKPLSGIGGEKTE--
*** ** *

Fig. 2 Amino acid alignment of fatty acid desaturases using standard one-letter amino acid codes. Identical residues are shown by star underneath. Identical residues to Tndes1 are shown on backgrounds of black. The underlined sequences are the histidine boxes. Tndes1, amino acid sequence encoded by Tndes1. Rape, Arath, Soybean and castorbean refer to the deduced amino acid sequence encoded by endoplasmic reticulum ω -3 desaturase from rapeseed (Arondel et al 1992), *Arabidopsis thaliana* (Yadav et al. 1993), soybean (Yadav et al. 1993) and plastid fad7 from castor bean (Van De Loo and Somerville et al. 1994) respectively.

SOSUI Result

Query title : Tndes1

Total length : 387 A. A.

Average of hydrophobicity : -0.260982

**This amino acid sequence is of a MEMBRANE PROTEIN
which have 4 transmembrane helices.**

No.	N terminal	transmembrane region	C terminal	type	length
1	57	RSLTYVFRDVVVFALAAAFYF	79	PRIMARY	23
2	172	RILRYSPLEPMFALPYLWRRS	193	SECONDARY	22
3	216	VLTSNFCFSIMALLLYSCFVFG	238	PRIMARY	23
4	243	LKFYGIPLYLVFVMWLDFTYMH	265	PRIMARY	23

12

Hydropathy profile

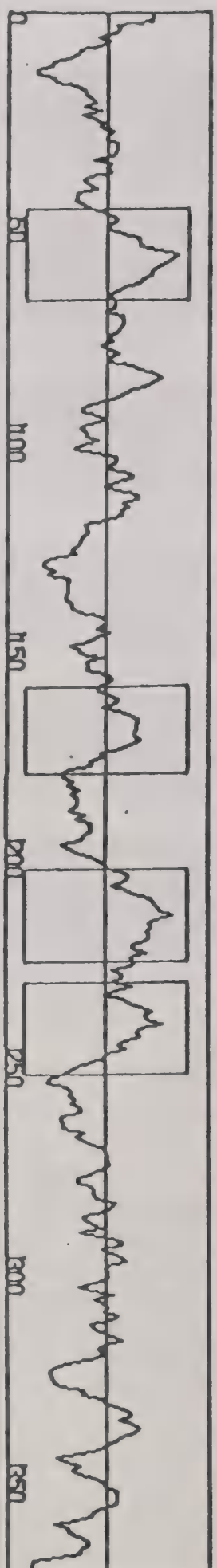


Fig. 3 Hydropathy profile of Tndes1 desaturase

*Survey of Fatty Acid Composition of Peanut and
Characterization of Its Epoxy and Eicosenoic Acids*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Survey of Fatty Acid Composition of Peanut and Characterization of Its Epoxy and Eicosenoic Acids

Earl G. Hammond^a, Daniel Duvick^a, Tong Wang^a, Hortense Dodo^b and R.N. Pittman^c

^aDepartment of Food Science and Human Nutrition and
Center for Crops Utilization Research, Iowa State University

^bDepartment of Food Science and Animal Industries, Alabama A&M University

^cUnited States Department of Agriculture-Agricultural Research Service, Griffin, GA

Introduction

Peanuts are known to contain significant amounts of C₂₀, C₂₂, and C₂₄ saturated fatty acids (1), and it has been suggested that the presence of these fatty acids on the sn-3 position of glycerol might account for the unusually high atherogenicity of peanut oil when it is fed with cholesterol (2). The dietary significance of this finding in humans has been questioned by Hayes et al. (3), but we wished to explore the possibility of breeding peanuts with reduced concentrations of the long-chain fatty acids. To this end, we analyzed the fatty acid composition of a core group of peanut plant introductions that had been selected to represent the variance in the peanut germplasm collection (4). The position of the double bond in eicosenoate of peanut has not been identified, although some tables of fat composition suggest that it is at Δ9 (1). Our preliminary analysis also showed additional unknown fatty acids in some peanuts. We located the double bond in the eicosenoate, and identified the unknown fatty acids.

Methods

- Peanuts obtained from the germplasm collection in Griffin, GA (grown in 1995)
- Fatty acid composition determined by GC with a 15-m DB-23 column, a method outlined by Hammond (5)
- For eicosenoate characterization, monoenoic fatty acid methyl ester (FAME) was separated from the other esters by silver ion thin layer chromatography (TLC). The extracted monoenoic ester was separated by C18 reverse-phase TLC. The 20:1 band was recovered. The sample was ozonized (6) and the ozonides converted to aldehyde with triphenylphosphine, and GC/MS was carried out with HP 5890 GC fitted with a 30-m SE-30 column and HP 5970 mass detector. Methyl *cis*-11-eicosenoate was used to aid the characterization.
- To identify epoxy fatty acid, total FAME was separated by TLC. Epoxy ester band was identified by comparing with methyl vernolate. The epoxy ester was reacted with acetic acid

and converted to dihydroxy derivatives with methanolic sodium methoxide. The sample was treated with *t*-butyldimethylsilylimidazol to obtain *t*-butyldimethylsilyl ethers, and GC/MS of the silyl ethers was performed. Pure methyl *cis*- and *trans*-9,10-epoxystearate were used to aid the identification.

Results

Peanut fatty acid distribution and correlation. The distributions of various fatty acids were examined. Palmitate and stearate show a tendency toward bimodal distributions. Oleate is skewed towards high values and linoleate to low values. Arachidate and eicosenoate are skewed to high values, but behenate and lignocerate are distributed normally. The total long-chain saturate, consisting of arachidate, behenate and lignocerate, is normally distributed and ranged from 3.7 to 10.2% with the mode at 6.5%. These results suggest it should be possible to obtain oils with a range of long-chain acyl group concentrations to verify that the atherogenic effect observed for peanut oil is caused by these acids. The ranges for fatty acid percentages found in the 732 lines included in this study exceeded the ranges reported previously except for that of Norden (7) where lines with high oleate and low linoleate exceeded the values found in this core collection.

Table 1 shows the correlations among the fatty acids. These correlations may reflect precursor-product relations in some instances but probably also reflect genetic linkages of various enzymes involved in the conversions. The strong negative correlation between oleate and linoleate results from their being the chief acyl groups in the oil so that one cannot increase much without a decrease in the other. All the saturated acyl groups are correlated with the saturated acyl group containing two more carbons, reflecting a precursor-product relation. Lignocerate is negatively correlated with palmitate, stearate and arachidate, whereas behenate is positively correlated with arachidate and lignocerate. Also oleate is negatively correlated with all the saturated acyl groups except lignocerate. Linoleate, on the other hand, is positively correlated with palmitate and behenate. The epoxyesters are positively correlated with palmitate and linoleate and negatively with oleate and eicosenoate.

Characterization of eicosenoic acid. GC/MS showed nonanal and methyl 11-oxoundecanoate as the ozonolysis products. Thus, the eicosenoate is gondoate (*cis*-11-eicosenoate), and gadoleate (*cis*-9-eicosenoate) is not present.

Identification of epoxy fatty acid. The group of-epoxy compounds were tentatively identified as *cis*-9,10-epoxystearate, *cis*-12,13-epoxyoctadec-9-enoate (vernolate), and *cis*-9,10-epoxyoctadec-12-enoate (coronarate), with an estimated ratio of 46:10:44. The nutritional

significance of the epoxy fatty acids in some peanut varieties is not clear. Maity and Mandel (8) attributed the poor growth response of rats fed *Acacia arabica* oil to its content of epoxy and hydroxy fatty acids.

Conclusions

- 732 peanut plant introductions were analyzed for fatty acid composition. Palmitate varied from 8.2 to 15.1%, stearate 1.1 to 7.2%, oleate 31.5 to 60.2%, linoleate from 19.9 to 45.4%, arachidate 0.8 to 3.2%, eicosenoate 0.6 to 2.6%, behenate 1.8 to 5.4%, and lignocerate 0.5 to 2.5%. The fatty acid percentages are related to each other.
- The eicosenoate was shown to be *cis*-11-eicosenoate.
- Epoxy fatty acids were found in many plant introductions in percentages ranging as high as 2.5%. These were tentatively identified as chiefly 9,10-epoxystearate and coronarate with smaller amounts of vernolate.

References

1. **White, P.J.**, Vegetable Oils, in *Fatty Acids in Foods and Their Health Implications*, edited by C.K. Chow, Marcel Dekker, New York, 1992, pp. 237-262.
2. **Myher, J.J., L. Marai, A. Kuksis, and D. Kritchevsky**, Acylglycerol Structure of Peanut Oils of Different Atherogenic Potential, *Lipids* 12:775-785 (1977).
3. **Alderson, L.M., K.C. Hayes, and R.J. Nicolosi**, Peanut Oil Reduces Diet-Induced Atherosclerosis in cynomolgus Monkeys, *Arteriosclerosis* 6:465-474 (1986).
4. **Holbrook, C.C., W.F. Anderson, and R.N. Pittman**, Selection of a Core Collection from the U. S. Germplasm Collection of Peanut, *Crop Sci.* 33: 859-861 (1993).
5. **Hammond, E.G.**, Rapid Analysis of Lipids in Many Individual Plants, in *Modern Methods of Plant Analysis*, New Series, V 12, edited by H.F. Liskens and J. F. Jackson, Springer-Verlag, New York. 1991, pp. 321-330.
6. **Hammond, E.G.**, Lipids and related compounds, in *Specifications and Criteria for Biochemical Compounds*, 3rd ed., National Academy of Sciences, Washington, DC, 1972, pp. 119-147.
7. **Norden, A.J., D.W. Gorbet, D.A. Knauff, and C.T. Young**, Variability in oil quality among peanut genotypes in the Florida Breeding Program. *Peanut Sci.* 14:7-11 (1987).
8. **Maity, C.R. and B. Mandal**, Chemical and Nutrition Studies on the Seed Oil of *Acacia arabica*, *J. Am. Chem. Soc.* 67:433-434 (1990).

Table 1. Correlations Among the Fatty Acids of 732 Peanut Plant Introductions^a

	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
18:0	0.19	--						
18:1	-0.58	-0.18	--					
18:2	0.40	-0.06	-0.93	--				
20:0	0.13	0.94	-0.21	-0.04	--			
20:1	-0.49	-0.79	0.19	0.01	-0.71	--		
22:0	0.06	0.22	-0.37	0.20	0.39	0.10	--	
24:0	-0.45	-0.45	0.07	-0.01	-0.30	0.73	0.41	--
Epoxy	0.34	0.07	-0.37	0.21	0.10	-0.16	0.10	-0.04

^aCorrelation coefficients $> \sim 0.1$ are significantly different from zero

*Phospholipid Fatty Acid Composition and
Stereospecific Distribution of Modified Soybeans*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8–10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Phospholipid Fatty Acid Composition and Stereospecific Distribution of Modified Soybeans

Tong Wang^a, Earl G. Hammond^a and Walter R. Fehr^b

^aDepartment of Food Science and Human Nutrition and the
Center for Crops Utilization Research

^bDepartment of Agronomy

Iowa State University

Ames Iowa 50011

Introduction

Modification of the fatty acid composition of soybean oil to make it more competitive in various segments of the food and industrial oil markets (1) has been an important objective of plant breeding and molecular genetics. Aside from the triglycerides (TGs), soybeans also contain phospholipids (PLs), with phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) being the major classes (2). These PLs are the major components of the cell membranes. It is important that these membrane PLs be in the proper physical state for cells to perform their metabolic tasks, and this physical state requires that the PLs have the correct balance of saturated and unsaturated fatty acids, as well as a balanced PL class composition (3).

PL compositional changes in the membranes of organisms in response to environmental temperature have been studied extensively, but information about the effect of genetic modification of soybean on PL fatty acid composition is very limited. In this study, soybean lines with the widest possible ranges of the five major fatty acids were analyzed for PL composition and stereospecific distribution to determine the effect of alteration in the overall fatty acid composition on the composition and distribution of PLs.

Experimental Procedures

Materials. Commercial soybean cultivars and experimental soybean lines were provided by the Agronomy Department at Iowa State University. Table 1 shows the typical fatty acid composition and the composition range selected for this study.

Lipid extraction and class separation. Duplicate samples of 2.00 g ground beans were extracted with chloroform:methanol (v:v, 2:1). Neutral and polar lipid class separation was achieved by solid phase extraction. To separate the major PL classes, total polar lipid solution was streaked on a thin-layer silica gel preparative plate and the plate was developed with chloroform:methanol:acetic acid:water (v:v:v:v, 100:45:5:2). PC, PE, and PI bands were scraped and recovered from the plate.

Stereospecific analysis. PL samples were hydrolyzed with snake venom (4). The sn-2 position fatty acid and lysoPL were separated by TLC using the same system used for PL class separation. All lipid fractions were converted to fatty acid methyl ester and analyzed by gas chromatography.

Results

PL content. Table 2 presents the average soybean lipid composition and relative proportions of the PLs. Crude total lipid percentage, PL percentage in crude lipid, total PL, and relative proportions of individual PL classes were correlated with TG fatty acid percentages (correlation coefficients are shown in Table 3 and Table 4) to explore the effect of oil composition on the quantity of PLs. There were significant positive correlations of %PE with total saturate and palmitate and a negative correlation with linoleate. There also was a negative correlation between %PI and palmitate. These results suggest that fatty acid composition of soybean affects PL class composition.

Relationship of PL and TG fatty acid composition. Fig.1 and Fig. 2 show the effect of changes in TG saturated fatty acid percentages on PL composition. With the increase of TG palmitate and stearate, all three PLs showed corresponding increases in these saturated esters. The slope of the PI plot was greater than those for PC and PE, suggesting that PI was the most sensitive PL to saturated fatty acid alteration. Note that for the PI palmitate plot, some points were well above and below the line. The group of points above the line had typical TG stearate level, whereas the

group below the line had elevated TG stearate. Similarly, for the PI stearate plot, the group of points above the line had decreased TG palmitate, and the group of points below the line had elevated TG palmitate. These deviations suggest that the presence of either of these saturates in PLs suppresses the incorporation of the other. The unsaturated fatty ester percentages of all PLs also were positively correlated with their TG percentages. All the slopes in the figures are less than one indicating that PL acyl group percentages change less than those for TG as acyl percentages vary.

PL fatty acid stereospecific distribution. Fig. 3 A, B, C, D, and E show plots for PC fatty ester percentages on the sn-1 and sn-2 positions as the total percentage of each fatty ester in the PL varies. The stereospecific distribution profiles of PE and PI are very similar to those of PC. Palmitate and stearate were predominantly located at the sn-1 position of all PLs. The changes of palmitate in PC, PE and PI were almost exclusively reflected on the sn-1 position. Unsaturated fatty esters had a different distribution profile than those of the saturates.

Conclusion

PL contained an average of 55.3% PC, 26.3% PE and 18.4% PI. PL class proportions were affected by changes in overall fatty acid composition. PL fatty acid composition changed with oil fatty acid modification, especially for palmitate, stearate and linolenate. Stereospecific analysis showed that saturated fatty acids were primarily located at the sn-1 position of all PLs, and changes of the saturates in PLs were largely reflected on this position. Oleate was distributed relatively equally between the sn-1 and sn-2 positions. Linoleate was much more concentrated on the sn-2 than on the sn-1 position for all PLs. Linolenate was distributed relatively equally at low concentration but preferred sn-2 position at high concentration.

Acknowledgment: This work was supported by research grant from Pioneer Hi-Bred International, Inc., Des Moines, IA.

References

1. **Hammond, E.G.**, Genetic alteration of food fats and oils, in *Fatty Acids in Foods and Their Health implications*, edited by C.K. Chow, Marcel Dekker, Inc., New York, 1992, pp.313-327.
2. **Hui, Y.H.**, Lecithins, in *Bailey's Industrial Oil and Fat Products*, vol. 1: *Edible Oil and Fat Product: General Application*, edited by Y.H. Hui, John Wiley & Sons, Inc., New York, 1996, pp.316-317.
3. **Chapman, D.**, Some recent studies of lipids, lipid-cholesterol and membrane systems, in *Biological Membranes*, vol. 2, edited by D.Chapman and D.F.H. Wallach, Academic Press, New York, 1973, pp.91-144.
4. **Robertson, A.F., and W.E.M. Lands**, Positional specificities in phospholipid hydrolyses, *Biochemistry* 1:804-810 (1962).

Table 1. Fatty acid composition range of 25 soybean lines evaluated, compared to the typical composition

	Palmitate 16:0	Stearate 18:0	Oleate 18:1	Linoleate 18:2	Linolenate 18:3
Typical, mole %	11	4	24	54	7
Range, mole %	3.1-33.3	2.4-24.2	7.8-35.3	35.3-68.3	2.7-16.3

Table 2. Average soybean lipid composition (25 lines)

% crude lipid in seed	23.7
% PLs in seed	0.9
% PLs in crude lipid	3.7
% PC of total PLs	55.3
% PE of total PLs	26.3
% PI of total PLs	18.4

Table 3. Correlations of total lipid and PL contents with various FA percentages in TG

	16:0	18:0	18:1	18:2	18:3
% total lipid	-0.60	-0.06	0.57	0.46	-0.56
% PL in total lipid	0.62	0.18	-0.49	-0.59	0.39
Total PL	0.35	0.20	-0.27	-0.41	0.15

Numbers in bold and italic are significant at 5%

Table 4. Correlations of PL class proportions with various FA percentages in TG

	16:0	18:0	18:1	18:2	18:3
PC %	-0.13	-0.10	-0.18	0.29	0.21
PE %	0.61	0.01	-0.09	-0.55	-0.04
PI %	-0.52	0.13	0.35	0.22	-0.24

Numbers in bold and italic are significant at 5%

Fig.1. PL palmitate as affected by oil palmitate

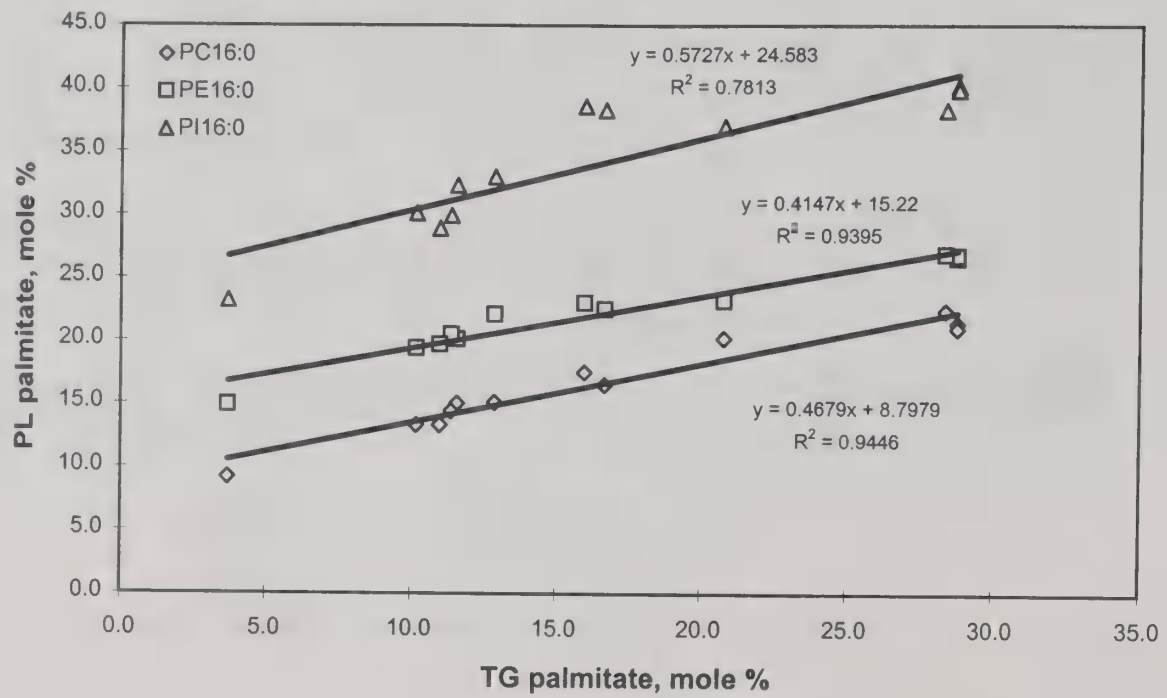


Fig.2. PL stearate as affected by oil stearate

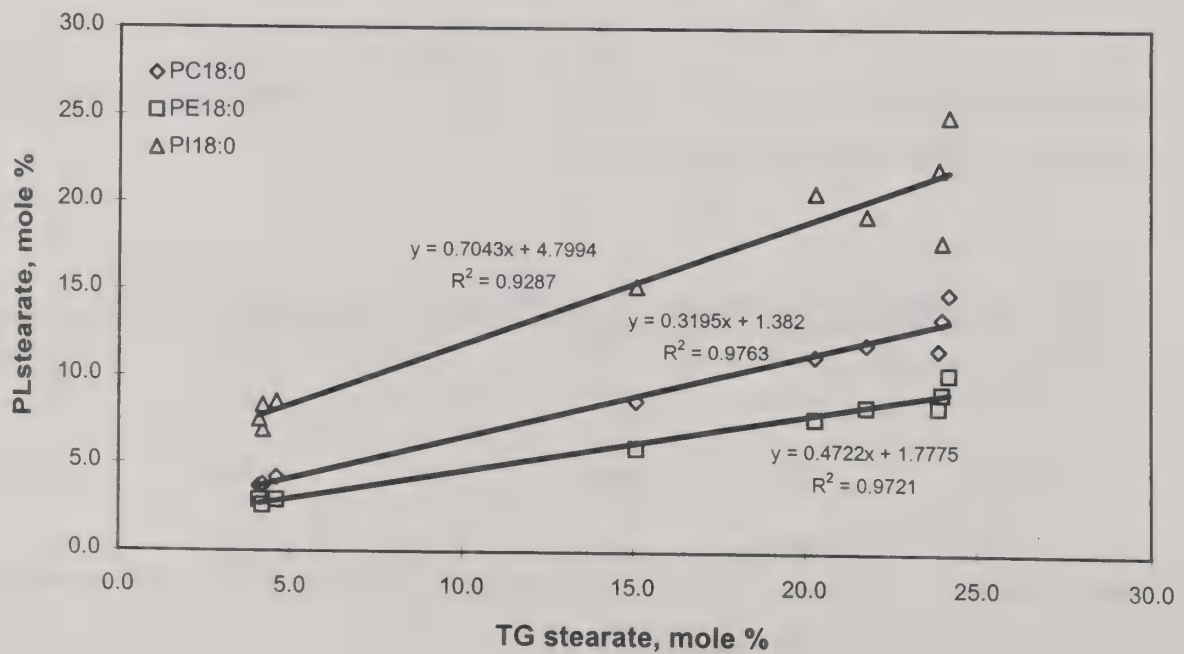


Fig. 3A. PC palmitate stereospecific distribution

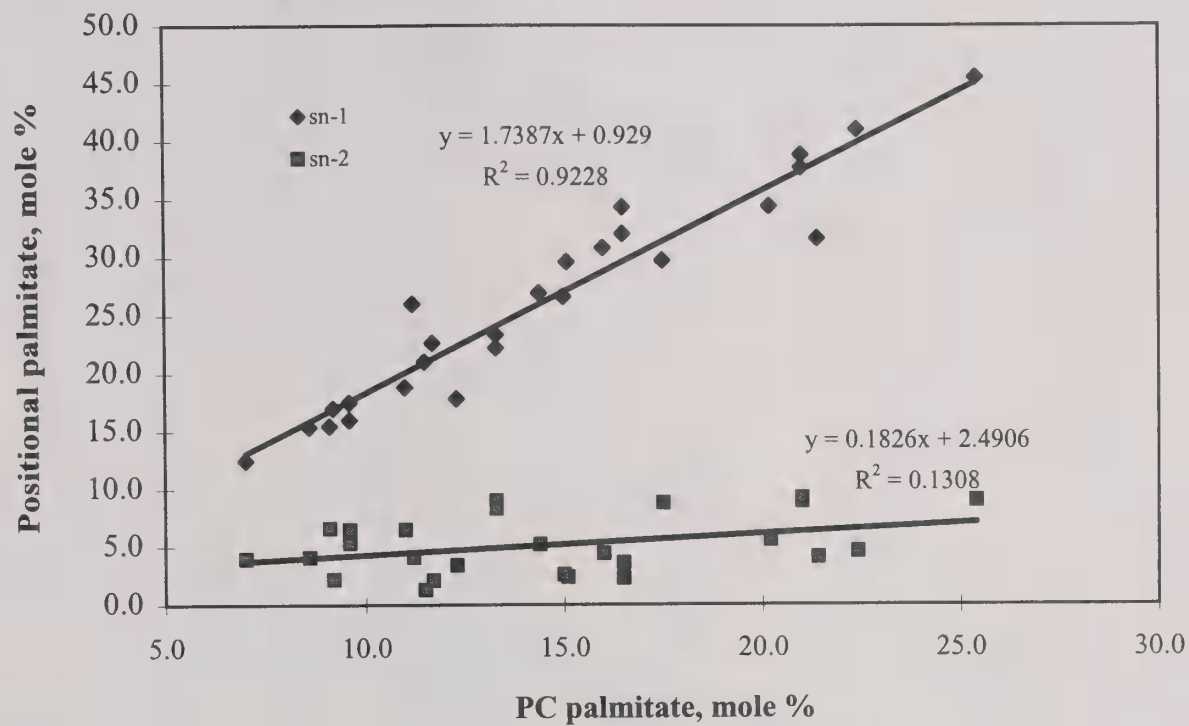


Fig. 3B. PC stearate stereospecific distribution

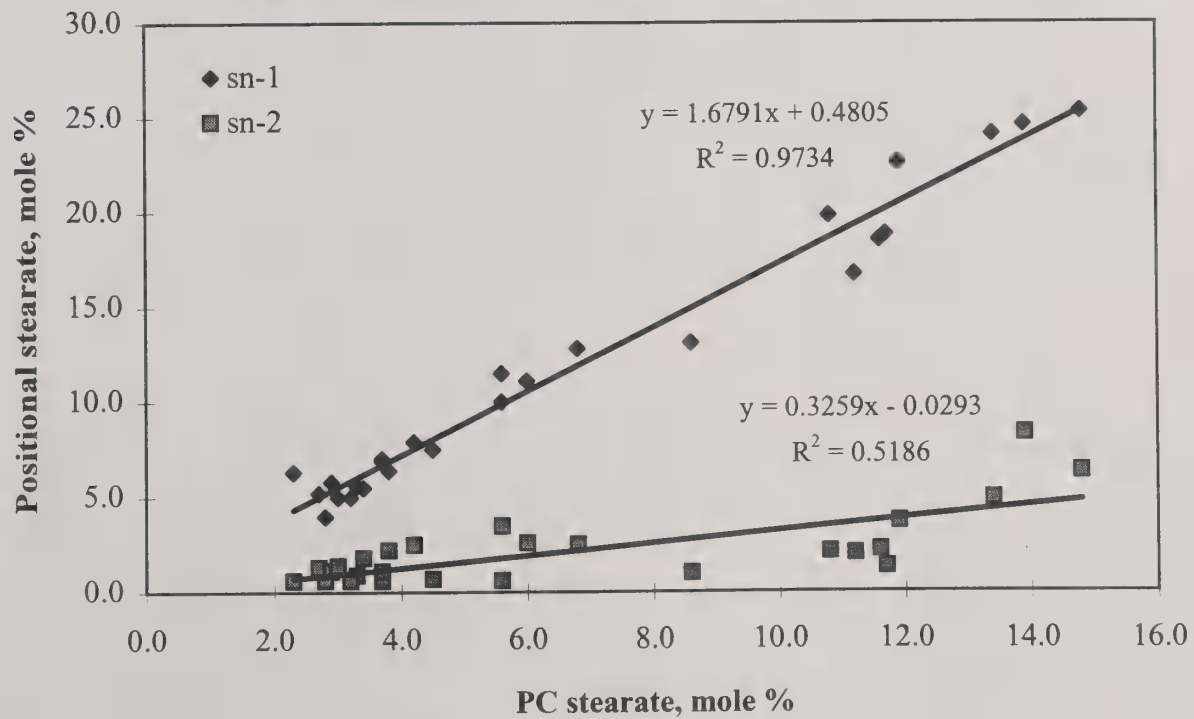


Fig. 3C. PC oleate stereospecific distribution

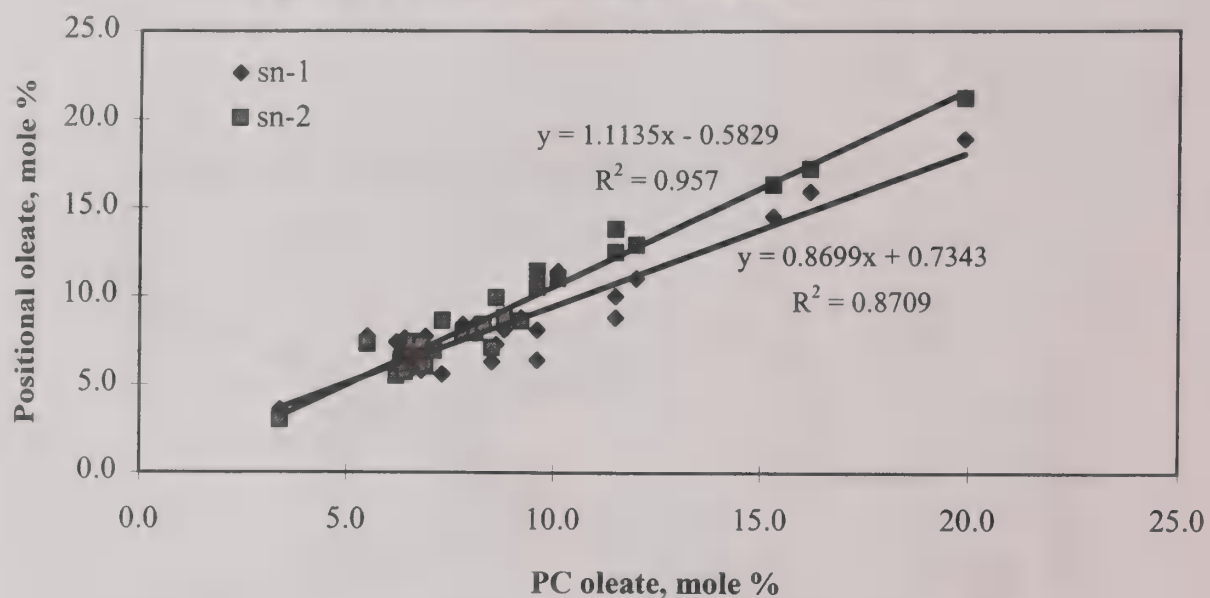


Fig. 3D. PC linoleate stereospecific distribution

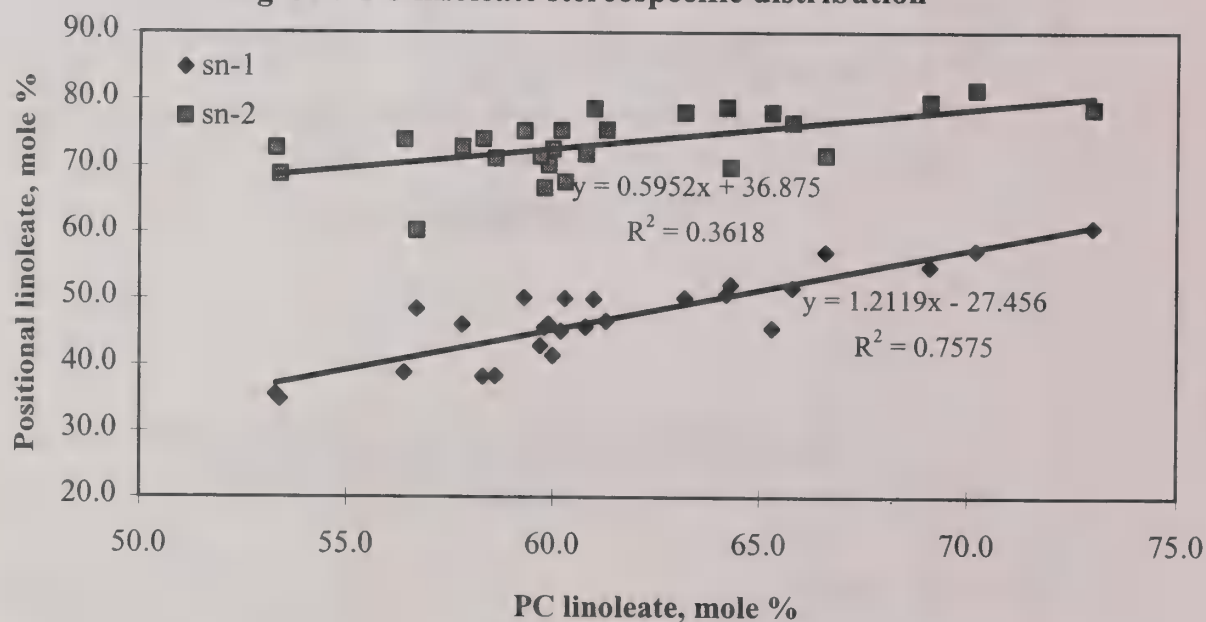
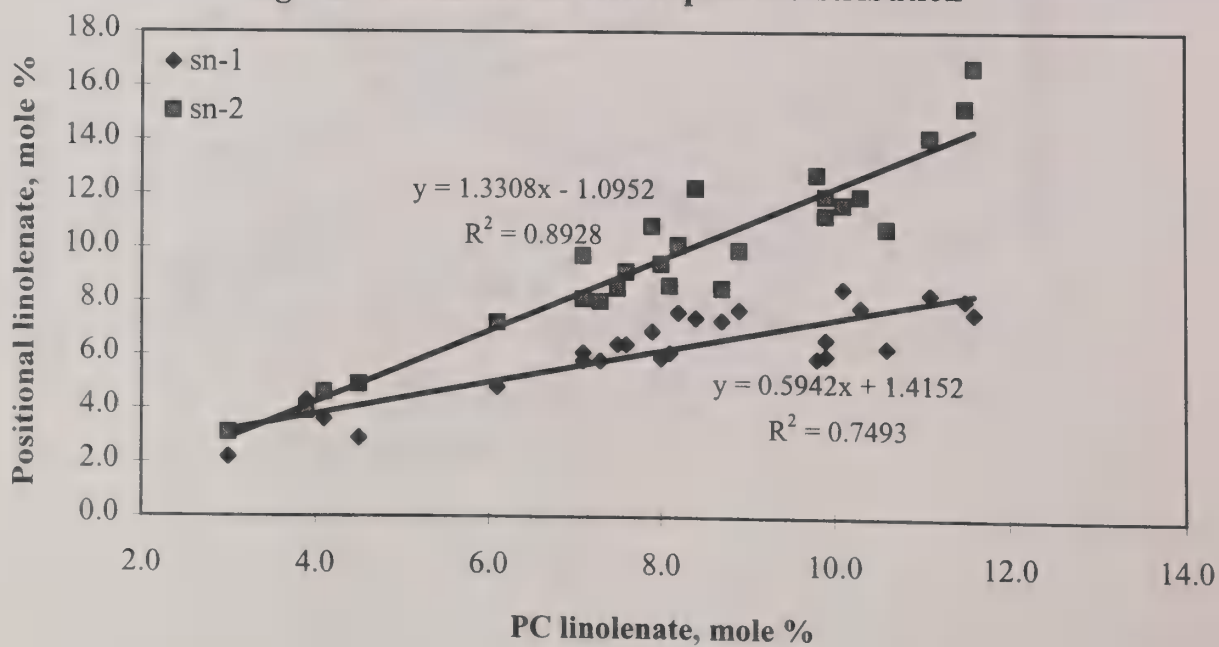


Fig. 3E. PC linolenate stereospecific distribution



*Research, Development and Technology Transfer
Activities of the Center for Crops Utilization Research*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

**Research, Development and Technology Transfer Activities
of the Center for Crops Utilization Research**

L. A. Johnson and D.J. Burden
Center for Crops Utilization Research
Iowa State University
1041 Food Sciences Bldg., Ames, IA 50011-1061

The Center for Crops Utilization Research focuses on expanding markets for Midwestern-grown crops by: developing new products and processes for corn, soybeans, and alternative crops to keep American agriculture competitive; replacing petroleum-derived products with those from renewable agricultural resources; channeling the application of biotechnology into expanding utilization; and transferring newly developed technologies to the private sector for commercialization. The center is interdisciplinary and is comprised of over 50 affiliated faculty from 18 academic departments of four colleges at Iowa State University. Over \$2,500,000 is attracted annually in research grants and contracts to support the center's program. The center coordinates newly constructed, highly specialized, pilot plant spaces and laboratories. In addition to typical food processing and product development and evaluation capabilities, the center has unique processing facilities in oil seeds' extraction, oil refining, wet milling, dry milling, brewing, degradable plastics, fermentation, and soy foods processing. The center also coordinates numerous workshops, conferences and seminars. This poster presentation will highlight the center's programs on corn and soybean oil extraction and oil refining, crambe seed meal detoxification, biodiesel production, specialty oils and meals from identity-preserved processing, and soy protein-based adhesives and plastics.

New Tools for Oilseed Processing Research



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

New Tools for Oilseed Processing Research

L. A. Johnson and M. A. Reuber
Center for Crops Utilization Research
Iowa State University
1041 Food Sciences Bldg., Ames, IA 50011-1061

A new pilot-plant extractor-simulator manufactured by the French Oil Mill Machinery Co. for use in soybean processing research was evaluated. This extractor-simulator uses about 40 lb of flakes, expanded collets or press cake and batch advances miscella and fresh solvent simulating designs of commercial extractors. The extractor can be run with bed depths ranging from 2 to 5 feet deep, and the equipment has provisions for measuring solvent flux through the extraction bed. When extracting soybean flakes in a counter-current mode with 5 stages of miscella (1.7:1 solvent:flakes ratio, equilibrium concentrations) and a final stage of fresh solvent, the residual oil contents were $0.68 \pm 0.27\%$ db. Residual oil contents vary by 0.25% db depending upon location depth in the bed. When using six stages of fresh solvent, the residual oil contents were $0.50 \pm 10\%$ db. Typical solvent flux rates for 5 ft beds of soybean flakes were 14.4 L/ft²/min. A three-deck desolventizer toaster can produce soybean meals with protein dispersibility indexes ranging from 30 to 90 depending upon desolventizing/toasting conditions. This extractor-simulator is uniquely designed to conduct extraction trials with genetically modified soybeans and to obtain limited quantities of oil and meal for applications testing. It is also useful in evaluating processing conditions on extraction rates and other important engineering parameters. Designs for matching oil refining capacity (4-5 L) will also be presented.

*Volatiles Produced During Deodorization
of Soybean Oil and Their Flavors Significance*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

TITLE: VOLATILES PRODUCED DURING DEODORIZATION OF SOYBEAN OIL AND THEIR FLAVOR SIGNIFICANCE

Authors: Jian-Wen Kao, Earl G. Hammond and Pamela J. White
Department of Food Science and Human Nutrition
And Center for Crops Utilization Research
2312 Food Sciences Building
Iowa State University, Ames, IA 50011
phone 515/294-3011
fax 515/294-8181
e-mail: pjwhite@iastate.edu

EXTENDED ABSTRACT

Freshly deodorized soybean oils have a nutty flavor considered to be acceptable by consumers. Sensory panel members looking for flavors caused by oxidation in soybean oils learn to discount this nutty flavor, but when the flavor intensity of freshly deodorized oils is compared with a standard, such as an odorless mineral oil, the freshly deodorized oil is seen to have a flavor intensity that can not be accounted for by recognized flavor volatiles arising from oxidation. Non-fat or reduced-fat foods often lack desirable flavors associated with fresh vegetable oils, such as soybean oils, and this observation may be partly related to the nutty flavor. It is likely that volatile compounds do not exist in large amounts in freshly deodorized oil because of the high vacuum and temperature treatments, therefore, little work has been done to examine the flavor compounds in freshly deodorized vegetable oil.

In this study we wished to see if we could account for the flavor of freshly deodorized soybean oil by examining the volatile flavor compounds generated during deodorization and by exploring the possible role of nonvolatile flavor compounds. Even though newly deodorized soybean oil has this characteristic nutty flavor, often no detectable head-space volatiles can be detected. Therefore, the cause of this flavor was

investigated by deodorizing soybean oil in an apparatus with a double cold trap that allowed the volatile compounds formed from the initial decomposition of hydroperoxides to be collected separately from those produced during the normal deodorization process. The chief volatile components from the normal deodorization process were hydrocarbons, which contributed little to no odor to the oil. The compounds having the greatest odor were carbonyls, especially heptanal and *cis*-4-heptenal. Table I lists the carbonyl compounds detected and their odor descriptions. Although these components should accumulate at some steady state concentration in an oil during its deodorization, none seemed to account for the flavor of the deodorized oil. By using a particle detector, it was shown that small particles could be generated in the human mouth that could provide a mechanism to bring oil with nonvolatile flavor components into contact with the olfactory organ. Attempts to separate possible nonvolatile flavors in deodorized oil from triacylglycerides by chromatography on alumina or reaction with 2,4-dinitrophenylhydrazine were unsuccessful. Possibly the flavor is caused by the glycerol esters themselves.

Table 1. Carbonyl compounds found in soybean oil

NO.	Compound	Retention time		Identification		Odor descriptor ^c
		(min)		MS ^a	RT ^b	
1	hexanal	6.15		+	+	green
2	<i>cis</i> -4-heptenal	9.54		+	+	fish oil
3	heptanal	9.72		+	+	heptanal
4	benzaldehyde	.21		+	+	cherry
5	2,4-heptadienal	12.96		+	+	fruity
6	nonanal	15.93		+	+	slight fruity
7	heptanoic acid	16.3		+	+	sweaty
8	phenylpropanone	17.27		+	+	fruity-rose
9	2-nonenal	17.32		+	+	aldehyde
10	3-methyl-2,4-nonadione	19.3		+	+	licorice
11	2,4-decadienal ^d	20.73	21.22	+	+	beany
12	decano- γ -lactone	24.3		+	+	buttery lactone
13	menadione	25.28		+	+	spicy
14	heptanal dimer	27.01		+	--	-----

^a MS: Mass Spectrometry^b RT: Retention Time^c Odor identified, within the retention time of each peak, by three trained, experienced sensory panelists^d *cis-cis* and *cis-trans* isomers elute at different times

*Development of Immunochemical Method
for the Assessment of Gossypol*



47th Oilseed Conference

World Class Manufacturing: Optimizing Plant Operations for Maximum Profit

March 8-10, 1998 • Doubletree Hotel • New Orleans, Louisiana, USA

Development of Immunochemical Method for the Assessment of Gossypol

Xi Wang and Leslie C. Plhak

Louisiana Agricultural Experiment Station

Louisiana State University

Baton Rouge, LA 70803

ABSTRACT

Gossypol's toxic and antinutritional nature to animals and human beings limits the use of cottonseed products. The traditional analysis methods of gossypol, colorimetric and HPLC, are not always correlated to whole animal toxicity and bioactivity analysis, because the existence of two enantiomeric forms of gossypol and the potential diversity of bound forms of gossypol. Antibodies to gossypol are being developed in our laboratory as tools for studying the "free" and "bound" gossypol fractions. Because of the relatively small size of gossypol, it must be bound to a protein carrier to be immunogenic and also bound to a different protein for use in the ELISA (enzyme-linked immunosorbent assay). Several methods are being investigated to obtain pure and characterized gossypol-protein conjugates including direct reaction (Schiff base formation) or using bifunctional linking arms (through phenolic groups of gossypol). MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) was found to be useful to measure the number of gossypol groups bound per protein molecule. Rabbits were immunized using gossypol-LPH (*Limulus polyphemus* hemolymph). The sera obtained after each boost were screened for anti-gossypol antibodies using an antibody-capture non-competitive ELISA. Signal versus background absorbances increased as the number of immunizations increased, indicating that anti-gossypol antibodies were produced. Methodology and results will be presented in this article.

INTRODUCTION

Gossypol (Figure 1) is a biologically active polyphenolic compound mainly found in cottonseed. It has gained a renewed interest in recent years for its potential value in the treatment of cancer (Gilbert et al., 1995; Hu et al., 1994), human immunodeficiency virus (Lin et al., 1993) and also as an antifertility agent (Chenoweth et al., 1994; Wu, 1989).

A number of investigations (Haschek et al., 1989; Rikihisa and Lin, 1989) have shown that gossypol is toxic to monogastric animals and also to young ruminants (Zelski et al., 1995). The most common toxic effect is cardiac irregularity which causes animal death (Cater, 1968). Gossypol can also be considered antinutritional because it inhibits some proteases such as pepsin and can limit the availability of amino acids (Finlay et al., 1973). Because of gossypol's toxic and antinutritional nature, the use of cottonseed products for animal feed or human use is limited.

The traditional analysis methods, such as colorimetric (Ba 8-78, 1987; Ba 7-58, 1987) and HPLC (Botsoglou, 1991; Hron et al., 1990), are not always correlated to whole animal toxicity methods (Kerr, 1989; Calhoun et al., 1990). This may be explained by the existence of two enantiomeric forms of gossypol having different biological activities (Gonzalez-Garza et al., 1992) or different forms of bound gossypol with different stabilities *in vivo* (Calhoun et al., 1990 and 1996).

Enzyme-linked immunosorbent assays (ELISA) are being widely adapted for qualitative and quantitative analyses. Immunoassays take advantage of high binding affinities between antigens and their corresponding antibodies, produced in an animal after immunization. A small molecule such as gossypol (MW 518) will not elicit an immune response unless first bound to a carrier protein. Advantages of ELISA include high specificity and high sensitivity. These characteristics make it possible to analyze crude extracts, possibly even insoluble analytes such as cottonseed matrix. Various antibody preparations, specifically recognizing different forms of bound gossypol, would be useful to improve our ability to measure these specific forms. This would improve our understanding of the relationship between gossypol form and bioactivity. This paper describes the production of protein-gossypol conjugates, immunization of rabbits and production of anti-gossypol polyclonal antibodies.

MATERIALS AND METHODS

Bovine serum albumin (BSA), *Limulus polyphemus* hemolymph (LPH), gossypol and AvidChrom-Protein A kit were purchased from Sigma Chemical Co. (St. Louis MO). Dialysis tubing was Cellulose Dialysis Sacks (MWCO 12,000) from Sigma Diagnostics (St. Louis MO). Flexible PVC Microtiter plates were from Dynatech Laboratories Inc. (Alexandria, VA). Immulon™ 1 and Immulon™ 2 HB plates were obtained from Dynex Technologies, Inc. (Chantilly, VA). Goat anti-rabbit IgG peroxidase conjugate was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA).

Phosphate-buffered saline (PBS) solution was prepared by dissolving 18 g NaCl, 2.22 g disodium hydrogen phosphate, 0.6 g potassium dihydrogen phosphate in 1.9 L of distilled water, the pH adjusted to 7.3 with 1N NaOH and the volume made up to 2 L with distilled water. For PBST, Tween 20 (1.0 g) was added before the pH was adjusted.

ABTS substrate solution was made by adding 10 mg 2,2'-Azino-bis(3-ethylbenthiazoline-6-sulfonic acid) (ABTS), 8 µl hydrogen peroxide (30%) solution in 24 ml pH 3.8 0.1M citrate buffer.

Preparation of Immunogen :

A scheme for the production of LPH-gossypol conjugates via Schiff base is shown in Figure 2. Two LPH-gossypol conjugates (LPH-G) were produced as follows:

LPH-G^A: Gossypol (23.5 mg) was dissolved in 6 ml methanol. LPH (24.5 mg) was dissolved in 6 ml PBS buffer, then the two solutions were mixed and reacted with continuous stirring for 48 h at 4 °C in the dark. The whole mixture was filtered through a Whatman No.1 filter paper and washed using ethyl ether to remove unreacted gossypol. The product collected on the filter was air dried and stored at 4 °C with desiccation.

LPH-G^B: Gossypol (24.5 mg) was dissolved in 23 ml methanol and mixed with 24.5 mg LPH dissolved in 11 ml PBS and reacted as described for LPH-G^A. After the reaction, the whole mixture was dialyzed in 1 L of 8 M urea for 24 h, 4 L of 50 mM ammonium carbonate for 24 h and finally 4 L of 25 mM of ammonium carbonate for 24 hours, then the product was lyophilized.

Immunization of Rabbits and Collection of Immune Sera:

Two female NZW rabbits (#16 and #15) were immunized with 1.0 mg of conjugate (LPH-G^A and LPH-G^B, respectively) in 2 ml of PBS/Freund's complete adjuvant (1:1). Rabbit #16 was first immunized at age 7 mo and rabbit #15 was immunized beginning at age 10 mo. Boosts were given monthly using the same concentration of conjugate in Freund's incomplete adjuvant. Blood samples were taken 2 weeks after each boost and transferred to a sterile vacutainer, allowed to clot 2 hours at room temperature, centrifuged at 12,000 rpm for 10 minutes. The sera were collected and tested for anti-gossypol antibodies. Preimmune sera were used as controls. Serum from rabbit #16 after the fourth boost was purified using an AvidChrom Protein A kit.

Preparation of Solid-Phase Conjugates:

BSA-gossypol conjugate (BSA-G) was produced via Schiff base (Figure 2) as follows: Gossypol (7.8 mg) was dissolved in 2 ml ethanol. BSA (50 mg) was dissolved in 25 ml PBS. These two solutions were mixed with 90 mg of sodium cyanoborohydride (NaCNBH₃) and reacted for 48 hours in the dark under N₂ at RT. After reaction, the mixture was centrifuged to remove the precipitate, then dialyzed in same way as described for LPH-G^B. To observe the effect of reducing agent, a conjugate was also prepared without the addition of NaCNBH₃.

Measurement of Gossypol:Protein in Conjugates:

The number of gossypol groups per BSA molecule were measured using Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).

Screening Sera for Anti-gossypol Antibodies:

An antibody capture ELISA was used to measure the presence of anti-gossypol antibodies in rabbit sera. Serial dilutions of BSA-G in PBS were prepared and added to the wells of a microtiter plate and incubated at 4 °C overnight (immobilization of solid-phase). The solutions were removed, each well filled with 120 µl/well 1% BSA in PBS and the plate was incubated 30 min at 37 °C (blocking). The solution was removed and then washed with

3 X 200 μ l of PBST, incubating each wash for 5 min at RT with shaking. Antisera or preimmune sera were diluted 1/1000 with 1% BSA in PBST and 50 μ l aliquots were added to the wells and incubated for 30 min at 37 °C. After washing, as described above, with 3 X 200 μ l of PBST, rabbit antibodies were detected by the addition 100 μ l of 1/10,000 diluted goat anti-rabbit IgG peroxidase conjugate, incubated for 30 min at 37 °C and washed. ABTS substrate was added (100 μ l/well) and peroxidase activity was measured as absorbance at 405 nm after 30 min of incubation at RT.

Optimization of ELISA:

Checkerboard ELISA was used to optimize [BSA-G] and [antisera]. Both [BSA-G] and [antisera] were varied and tested in the antibody-capture ELISA described above. The highest concentration of either variable that showed an increase in absorbance was chosen as optimal and used for further analyses. Microtiter plates were compared for their ability to immobilize solid phase (maximum signal) and resistance to high background absorbance (minimum noise). The effect of antibody purification was tested using a Protein A kit.

RESULTS

Reaction of Gossypol with Protein:

The reaction between aldehyde groups in gossypol and amine groups of protein involves the formation of Schiff base intermediates. This reaction is reversible in aqueous solution (Thermanson, 1995). Addition of NaCNBH₃, a reducing agent, can convert the Schiff base to a secondary amine, which is more stable (Figure 2). MALDI-MS results for BSA-G made either with or without the addition of NaCNBH₃, showed 3 and 6 groups of gossypol per BSA, respectively, indicating that the reaction is favored in the presence of reducing agent.

Production of Antibodies:

The progress of immunization was monitored using an antibody-capture ELISA. Figure 3 shows the increase in absorbance over the immunization period for rabbit #16. The increase reflects either greater concentration or binding affinity of anti-gossypol

antibodies in the rabbit serum over time. Using a checkerboard ELISA (Figure 4), the optimal concentration of solid-phase conjugate was found to be 10 $\mu\text{g/mL}$ and the optimal serum dilution (for rabbit #16 after four boosts) was determined to be 1/1000. The type of microtiter plates used for the ELISA were found to have a great effect on the results (Figure 5). Immulon™ 2 was found to have the highest absorbance for immune sera and lowest background absorbance for preimmune sera when compared to Immulon™ 1 or flexible PVC plates. Purification of IgG antibodies using Protein A had little effect on the performance of the antibodies.

DISCUSSION

The stabilization of gossypol-protein (aldehyde-amino) complexes by the reducing agent NaCNBH_3 could explain why ruminant animals are less susceptible to gossypol toxicity than monogastric animals. The rumen is a highly reducing environment, having a redox potential of -0.4 volts (Brock et al., 1994). This could favor stabilization of gossypol-protein Schiff base intermediates, reducing the bioavailability of gossypol when cottonseed is fed to ruminants. Gossypol bound by a different mechanism may not be stable in the rumen. Gossypol contains two types of potentially reactive functional groups, aldehydes and phenyls. Different mechanisms of gossypol conjugation (to protein or other components in the cottonseed matrix) may explain why gossypol content determined using the AOCS method does not always correlate with animal feed bioavailability (Calhoun et al., 1990 and 1996).

Polyclonal antibodies were successfully produced for gossypol when rabbits were immunized repeatedly with a protein-gossypol conjugate produced via Schiff base intermediate. It was demonstrated that even a protein-reactive molecule such as gossypol can elicit antibodies. Current efforts in our laboratory are aimed at defining the specificity of the antibodies produced, developing monoclonal antibodies to specific forms of bound gossypol and adapting anti-gossypol antibodies to a competitive ELISA format for quantitation of gossypol.

ACKNOWLEDGMENTS

The authors acknowledge financial support from Cotton Incorporated (Raleigh, NC), Cooperative Agreement No. 96-401.

REFERENCES

- Botsoglou, N.A. 1991. High-performance liquid chromatographic method for the determination of free gossypol in chicken liver. *J. Chromatog.* 578:333-337.
- Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. 1994. In: *Biology of Microorganisms*. Prentice-Hall, Inc., Englewood Cliffs, NJ. pp. 655.
- Calhoun, M. C., Huston, J.E., Baldwin, B.C. Jr., Kuhlmann, S.W., Engdahl, B.S. and Bales, K.W. 1990. Effects of cottonseed meal source and dietary crude protein on performance of early-weaned lambs: with observations on gossypol toxicity. In: *Sheep and Goat, Wool and Mohair, 1990 Research Reports*. PR 4790. Texas A & M University System, College Station, TX.
- Calhoun, M. C. 1996. Safe levels of cottonseed for cattle. In: *Cotton Incorporated Agricultural Research Reports. Summary Reports 1996*. Cotton Incorporated, Raleigh, NC.
- Cater, C.M. 1968. Studies on the reaction products of gossypol with amino acids, peptides, and proteins. Dissertation. Texas A & M University.
- Chenoweth, P.J., Risco, C.A., Larsen, R.E., Velez, J., Tran, T. and Chase, C.C. Jr. 1994. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. *Theriogenol.* 42:1-13.
- Finlay, T. H., Dharmgrongartama, E.D. and Perlmann, G.E. 1973. Mechanism of the gossypol inactivation of pepsinogen. *J. Bio. Chem.* 13:4827-4833.
- Gilbert, N.E., O'Reilly, J.E., Chang, G., Lin, Y.C. and Brueggemeier, R.W. 1995. Antiproliferative activity of gossypol and gossypone on human breast cancer cells. *Life Sci.* 57(1):61-67.
- Gonzalez-Garza, M.T., Matlin, S.A., Mata-Cardenas, B.D. and Said-Fernandez, S. 1992. Further studies on the *in vitro* activity of gossypol as antiamebic agent. *Arch. Med. Res.* 23(2):69-70.
- Haschek, W.M., Beasley, V.R., Buck, W.B. and Finnell, J.H. 1989. Cottonseed meal (gossypol) toxicosis in a swine herd. *JAVMA.* 195(5):613.
- Hermanson, G.T. 1995. Zero-length cross-linkers. In: *Bioconjugate Techniques* Hermanson, G.T. (Ed.), p.185, Pierce Chemical Company, Rockford, IL.
- Hrons, R.J. Sr., Kuk, M.S. and Abraham, G. 1990. Determination of free and total gossypol by high performance liquid chromatography. *JAOCs.* 67(3):182-187.
- Hu, Y-F., Chang, C.J.G., Brueggemeier, R.W. and Lin, Y.C. 1994. Presence of antitumor activities in the milk collected from gossypol-treated dairy cows. *Cancer Lett.* 87:17-23.
- Kerr, L.A. 1989. Gossypol toxicosis in cattle. *Comp. Cont. Educ. Prac. Vet.* 11:1139.
- Lin, T.S., Schinazi, R.F., Zhu, J., Birks, E. and Carbone, R. 1993. Anti-HIV-1 activity and cellular pharmacology of various analogs of gossypol. *Biochem. Pharmacol.* 46(2):251-255.

- Official Method. American Oil Chemists' Society. 1987, Ba 7-58.
- Official Method. American Oil Chemists' Society. 1987, Ba 8-78.
- Rikihisa, Y. and Lin, Y.C. 1989. Effects of gossypol on the thyroid in young rats. *J. Comp. Path.* 100:411-417.
- Rosenberg, L.J., Adlakha, R.C., Desai, D.M. and Rao, P.N. 1986. Inhibition of DNA polymerase alpha by gossypol. *Biochem. Biophys. Acta.* 866:258-267.
- Wu, D.F. 1989. An overview of the clinical pharmacology and therapeutic potential of gossypol as a male contraceptive agent and in gynecological disease. *Drugs.* 38:333.
- Zaidi, R. and Hadi, S.M. 1992. Complexes involving gossypol, DNA and Cu(II). *Biochem. Intl.* 28(6):1135-1143.
- Zelski, R., Rothwell, J., Moore, R. and Kennedy, D. 1995. Gossypol toxicity in preruminant calves. *Australian Vet. J.* 72(10):394-398.

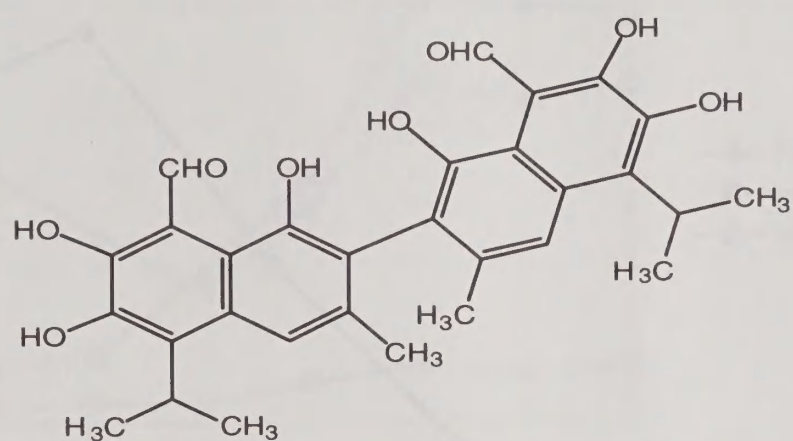


Figure 1. Gossypol

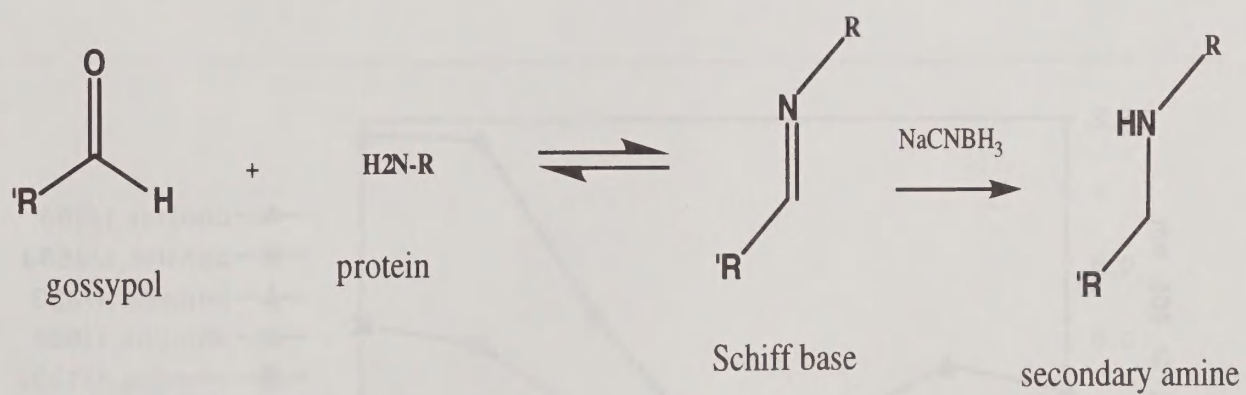


Figure 2. Production of BSA-gossypol through a Schiff base intermediate.

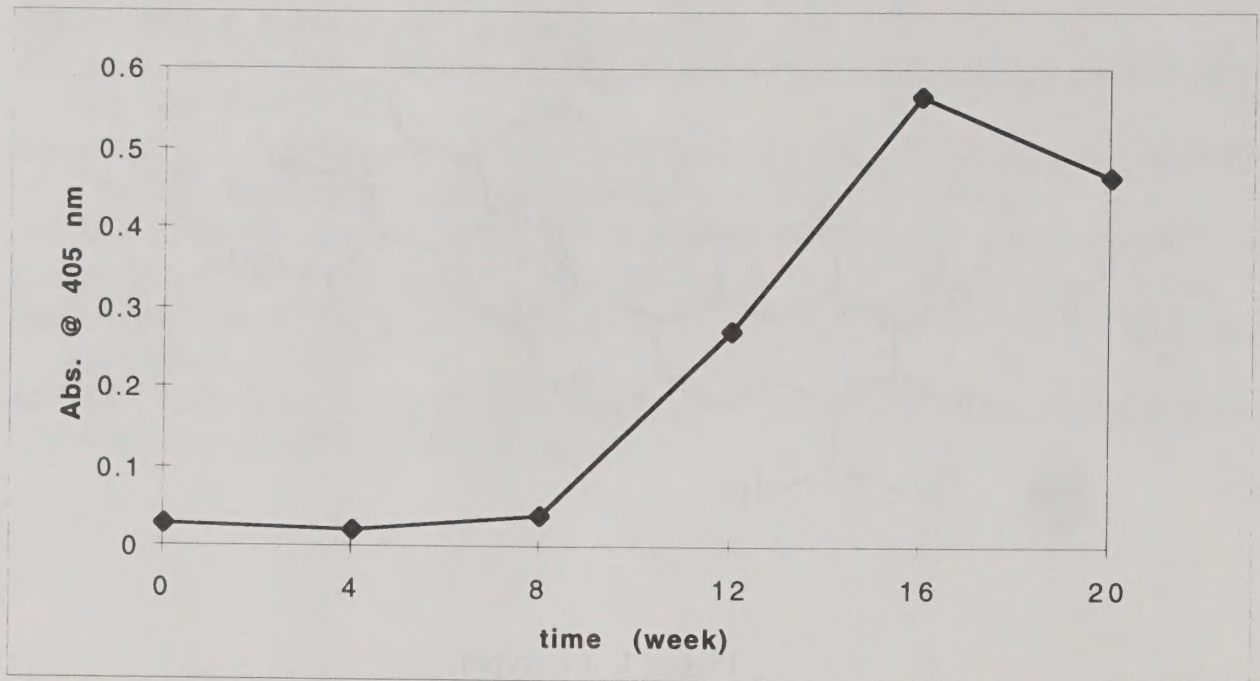


Figure 3. Anti-gossypol antibody production during immunization period for rabbit #16. Boosts were made at 2, 6, 10, 14 and 18 weeks. Sera were diluted 1/1000 and [BSA-G] was 10 $\mu\text{g/mL}$.

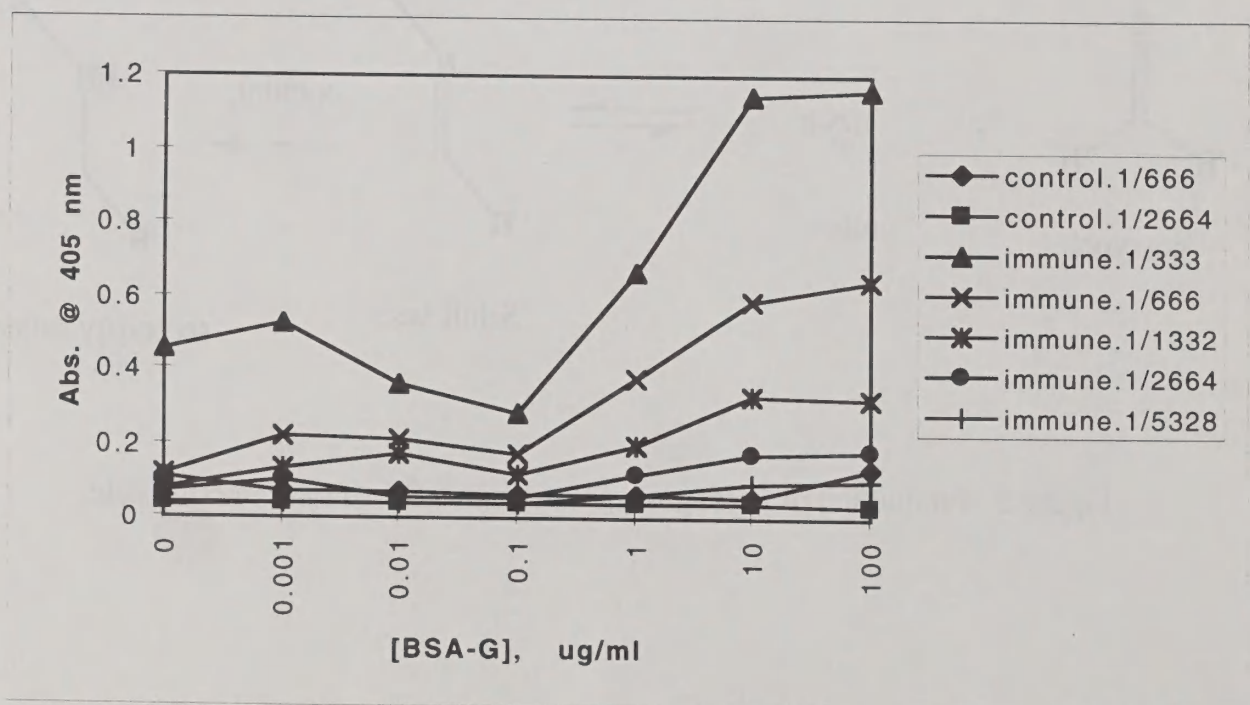


Figure 4. Effect of [BSA-G] and immune serum dilution on ELISA results. Immune serum was from rabbit #16 after four boosts. Control was preimmune serum.

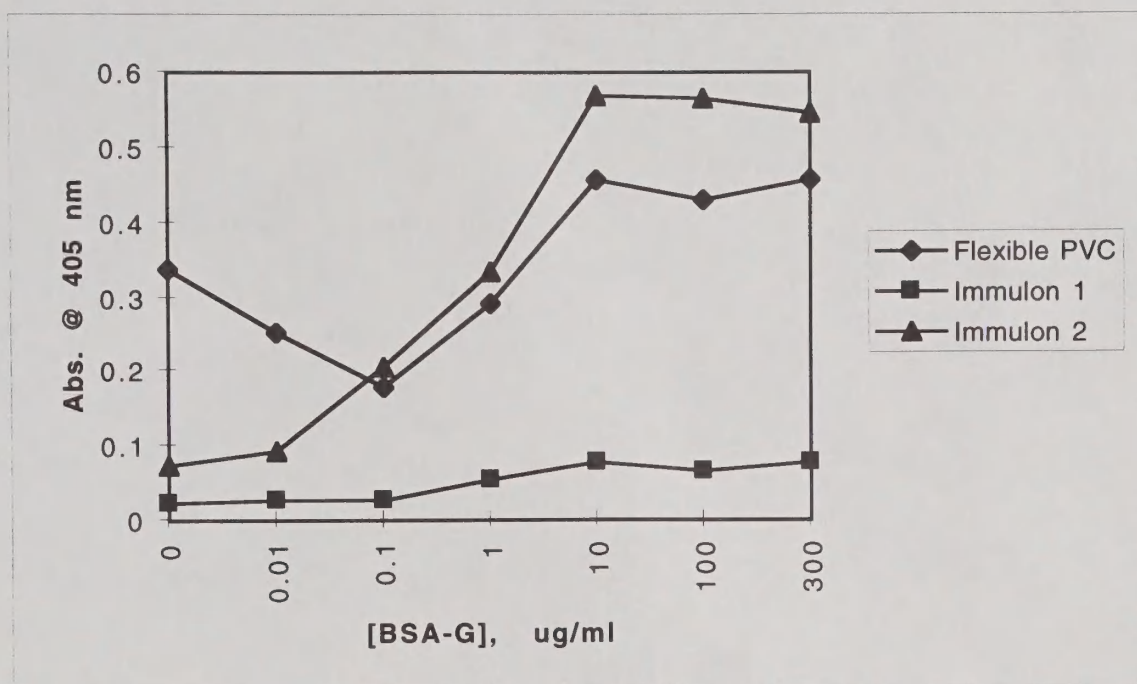


Figure 5. Comparison of different microtiter plates.

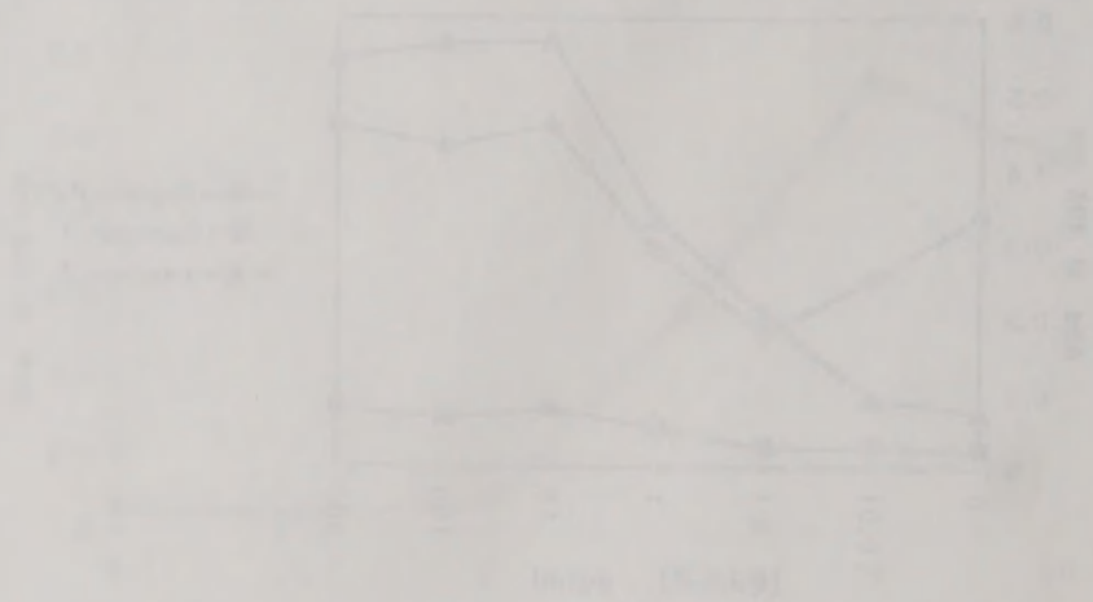


Figure 2. Effect of pH on the stability of the solution. The solution was prepared by dissolving 1.0 g of the polymer in 100 mL of water. The pH was adjusted by adding 0.1 M HCl or 0.1 M NaOH. The stability was measured by the change in viscosity over time.

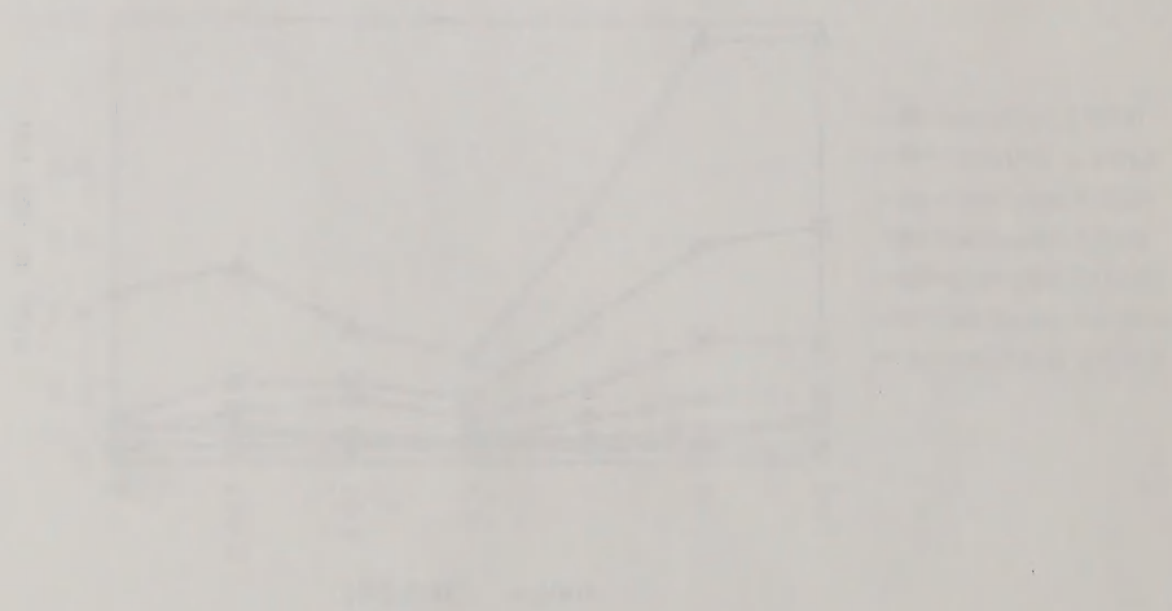


Figure 3. Effect of temperature on the stability of the solution. The solution was prepared by dissolving 1.0 g of the polymer in 100 mL of water. The temperature was adjusted by adding ice or heating. The stability was measured by the change in viscosity over time.